The Synthesis of Some 5-Methoxypyrimidine Nucleosides

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The synthesis of 5-methoxyuridine (**3**), 5-methoxycytidine (**6**), 1-(2-deoxy-β-D-*erythro*pentofuranosyl)-5-methoxyuracil (**14**), 5-methoxy-1-β-D-ribofuranosyl-4-thiopyrimidin-2-one (**5**), 1-β-D-arabinofuranosyl-5-methoxycytosine (**12**), 1-β-D-arabinofuranosyl-5-methoxyuracil (**8**) and 1-β-D-arabinofuranosyl-5-methoxy-4-thiopyrimidin-2-one (**11**) have been accomplished. Both **3** and **14** were synthesized by alkylation of 2,4-*bis*(trimethylsilyl)-5-methoxyuracil (**1**) with the appropriately blocked halosugars. Synthesis of the corresponding 5-methoxy-1-β-D-arabinofuranosyl derivatives was accomplished through the intermediate 2,2′-anhydro-1-β-D-arabinofuranosyl-5-methoxyuracil (**7**). The cytosine and 4-thiouracil derivatives in both the arabino- and ribo- series were prepared by thiation followed by amination.

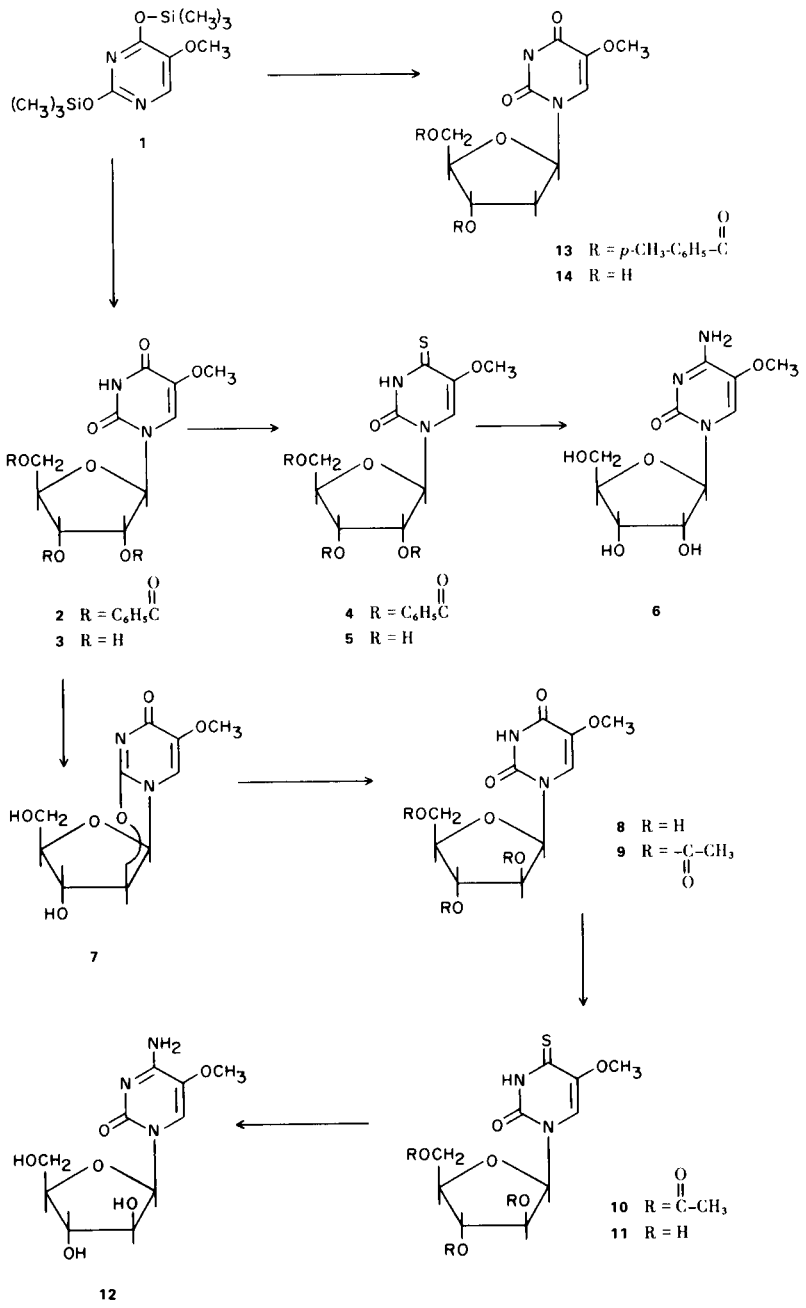
5-Iodo-2′-deoxyuridine (5-IdU) exhibits anti-herpes activity (1). Its mode of action has been shown to be by competitive inhibition of enzyme systems concerned with virus-DNA synthesis such as thymidine kinase and thymidylate synthetase (2). 5-Methylamino-2′-deoxyuridine has also exhibited good anti-herpes activity *in vitro* (3).

Several other 5-substituted uridine derivatives have shown marked pharmacological activity. 5-Ethyluridine has been shown to inhibit the growth of the thymine-deficient mutant of *E. coli* (4). 5-Mercaptouridine, 5-mercapto-2′-deoxyuridine and 5-methylthio-2′-deoxyuridine have been prepared by Bardos and co-workers (5). 5-Mercaptouridine has significant inhibitory activity against *Streptococcus faecalis* (6), and 5-mercapto-2′-deoxyuridine is reported (7) to sensitize cancer cells and then eradicate the sensitized cells in superficial basal cell epithelioma. 5-Methylthio-2′-deoxyuridine has shown a virus rating (V.R.) of 0.8-1.2 (8) against type 1 Herpes simplex in our laboratory. In view of these data the synthesis of the previously unknown nucleosides of 5-methoxyuracil and 5-methoxycytosine was undertaken.

5-Methoxyuracil was prepared by the method of Chesterfield (9). Conversion to the trimethylsilyl derivative was accomplished in 96% yield by refluxing the 5-methoxyuracil in hexamethyldisilazane using ammonium sulfate as catalyst. The resulting *bis*(trimethylsilyl)-5-methoxyuracil was alkylated with 2,3,5-tri-*O*-benzoyl-D-ribofuranosylbromide(**10**) in dry acetonitrile (11) to give a 75% yield of 5-methoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-uracil (**2**). Tlc analysis of the reaction mixture gave no indication of a second spot which could have been the α-anomer. The benzoyl groups on **2** were removed with a catalytic amount of sodium methoxide in methanol at room temperature for three days. The yield in the deblocking step was 95%. The site of alkylation was established as *N*-1 by comparison of the uv spectrum of **3** with the uv spectra of 5-methoxy-N^1-methyluracil and 5-methoxy-N^3-methyluracil (12). These uv spectral data (bathochromic shift of ~20 nm with *N*-3 alkyl derivatives in alkali) are consistent with the spectral trend of *N*-1 and *N*-3 alkylated pyrimidines reported by Winkley and Robins (13). The benzoyl blocked derivative **2** was thiated with phosphorous pentasulfide in pyridine at reflux to give 5-methoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4-thiopyrimidin-2-one (**4**). The product **4** was obtained as fleecy-yellow needles in 91% yield. Debenzoylation of **4** proved to be somewhat difficult in that **4** was not soluble in methanol, containing a catlytic amount of sodium methoxide, even at reflux. In order to achieve solution tetrahydrofuran was added to the methanol-sodium-mixture and then removed by distillation after solution had taken place. Methanol was added to maintain the original volume until the tetrahydrofuran was gone and then the resulting solution was refluxed for 10 hours to obtain complete removal of the three benzoyl groups.

An 84% yield of 5-methoxycytidine (**6**) was achieved by heating **4** in methanolic ammonia at 93° in a sealed container for 21 hours. The vessel was cooled, opened and the solvent was removed *in vacuo*. The resulting gum was crystallized from an ethanol-methanol mixture.

The facile epimerization at 2′ first described by Hampton (14) and explored in our laboratory (11) as a synthetic route to pyrimidine-β-D-arabinosides was utilized. A mixture of 5-methoxyuridine (**3**), diphenylcar-



bonate and sodium bicarbonate in dimethylformamide was heated at 150° for 30 minutes to give an 83% yield of 2,2′-anhydro-1-β-D-arabinofuranosyl-5-methoxyuracil (**7**). The anhydro bond of **7** was opened in 79% yield by heating a solution of **7** in dilute base for 1¼ hours. 1-β-D-Arabinofuranosyl-5-methoxyuracil (**8**) was readily acetylated in pyridine with acetic anhydride but the acetyl derivative (**9**) would not crystallize. The foam (**9**) was thiated directly in pyridine with phosphorous pentasulfide to give 5-methoxy-4-thio-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)pyrimidin-2-one (**10**) which was deacetylated with a catalytic amount of sodium methoxide in methanol to give 1-β-D-arabinofuranosyl-5-methoxy-4-thiopyrimidin-2-one (**11**).

Another quantity of the acetyl thione (**10**) was converted to 1-β-D-arabinofuranosyl-5-methoxycytosine (**12**) by bubbling ammonia slowly through a solution of **10** in methanol. The reaction was held at 45° and required 7 days to reach completion. Methanol was added periodically to replenish that which had evaporated and the progress of the reaction was followed by monitoring the shift in the uv spectrum from 355 nm to 295 nm.

The condensation of 2,4-*bis*(trimethylsilyl)-5-methoxyuracil (**1**) and 2-deoxy-3,5-di-*O*-*p*-tolyl-D-*erythro*pentofuranosyl chloride (15) was carried out in dry acetonitrile to give a product which could not be separated into anomers by tlc. The product (**13**), after repeated attempts at purification by fractional crystallization, still appeared to be impure (melting range of 12°). Deblocking of **13** was accomplished in methanol containing a catalytic amount of sodium methoxide and enough tetrahydrofuran to achieve solution. After removal of the tetrahydrofuran by distillation the methanol solution was refluxed for 10 hours, cooled to room temperature, neutralized with Dowex 50 (H^+), filtered and the solvents removed *in vacuo*. Crystallization of the resulting solid from methanol gave a 31% yield of 1-(2-deoxy-β-D-*erythro*pentofuranosyl)-5-methoxyuracil (**14**) (2′-deoxy-5-methoxyuridine). The anomeric configuration of the nucleoside was assigned on the basis of pmr spectral analysis. The anomeric proton exhibited a "pseudo-triplet" at J = 6.23 Hz and peak width of 13 Hz, indicating the *beta* configuration (16).

The 5-methoxypyrimidine nucleosides were devoid of significant activity against Herpes simplex virus in tissue culture.

EXPERIMENTAL

Infrared spectra were determined on a Perkin-Elmer 257 spectrophotometer (potassium bromide), ultraviolet spectra on a Cary 15 spectrophotometer and pmr on a Perkin-Elmer R-20 using TMS as internal standard. Tlc was done on Brinkman silica gel plates using ethyl acetate-propanol-water (4:1:2, upper phase) solvent system unless otherwise noted. C, H and N analyses were determined by M-H-W Laboratories, Garden City, Michigan.

2,4-*Bis*(trimethylsilyl)-5-methoxyuracil (**1**).

A mixture of 50 g. of 5-methoxyuracil (**9**) and 50 mg. of ammonium sulfate in 150 ml. of hexamethyldisilazane (HMDS) was refluxed gently for 18 hours. Complete solution had taken place after 2 hours. The excess HMDS was removed under aspirator vacuum and the residue was then distilled under high vacuum. The bath was 155-165° and the b.p. 103-104° at 0.6 mm. Hg. The yield was 96.5 g. (96%) of colorless oil which solidified on standing.

5-Methoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)uracil (**2**).

2,3,5-Tri-*O*-benzoyl-D-ribofuranosyl bromide from 53 g. of (0.105 mole) of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose was dissolved in 350 ml. of dry acetonitrile and 25.7 g. (0.09 mole) of 2,4-*bis*(trimethylsilyl)-5-methoxyuracil (**1**) was added. The solution was initially cloudy but cleared as it stirred. After 24 hours solid had begun to form and upon termination of the reaction after 45 hours, the mixture was nearly solid. The cake was broken up and the acetonitrile removed by evaporation. The residue was heated to boiling in 500 ml. of ethanol and 100 ml. of water was added. The reaction mixture was cooled, filtered and the solid dried to give 39.5 g. (75%) of **2** which melted at 208-210°. Crystallization from ethanol-methanol (1:1) gave material which melted at 210-212°.

Anal. Calcd. for $C_{31}N_{26}N_2O_{10}$: C, 63.48; H, 4.47; N, 4.78. Found: C, 63.23; H, 4.23; N, 4.63.

5-Methoxyuridine (**3**).

A solution of 20 g. of 5-methoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)uracil (**2**) and 1.5 g. of sodium methoxide in 2 l. of methanol was left at room temperature. After 3 days an additional 0.5 g. of sodium methoxide was added. The reaction was allowed to remain an additional 6 hours and then neutralized with Dowex 50 (H^+). The solution was evaporated to *ca.* 400 ml., cooled and filtered to give 7.8 g. of **3**. A second crop of 1.05 g. of **3** was obtained by evaporation of the mother liquor, trituration with ether to remove methyl benzoate and crystallization of the product from methanol to give a 95% yield. A sample was crystallized from methanol for analysis, m.p. 220-223°; uv: λ max (*p*H 1) 279 nm (8,600) λ max (*p*H 11) 277 nm (7,100).

Anal. Calcd. for $C_{10}H_{14}N_2O_7$: C, 43.80; H, 5.15; N, 10.22. Found: C, 43.89; H, 5.10; N, 9.94.

5-Methoxy-4-thio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidin-2-one (**4**).

A solution of 10 g. of 5-methoxy-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)uracil (**2**) and 10 g. of phosphorus pentasulfide in 120 ml. of pyridine was refluxed gently for 5 hours. Excess pyridine (*ca.* 30 ml.) was removed under vacuum. The hot mixture was then poured into 700 ml. of water at 60° with rapid stirring. The aqueous solution was cooled overnight, filtered, the solid washed with water and dried. The solid was boiled in 400 ml. of methanol-ethanol (1:1) for ½ hour, then cooled, filtered and dried to give 9.3 g. (91%) of **4**. Recrystallization from a benzene-ethanol mixture gave fleecy yellow needles which melted at 223-224.5°.

Anal. Calcd. for $C_{31}H_{26}N_2O_9S$: C, 61.78; H, 4.35; N, 4.65. Found: C, 61.81; H, 4.26; N, 4.61.

5-Methoxy-4-thiouridine (**5**).

To a solution of 350 mg. of sodium methoxide in 300 ml. of methanol and 80 ml. of tetrahydrofuran was added 3.14 g. of 5-methoxy-4-thio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidin-2-one (**4**). The tetrahydrofuran was distilled and the remaining solution was then refluxed for 10 hours. The solution was neutralized with Dowex 50 (H^+), filtered and concentrated to dryness. The residue was triturated with chloroform. The remaining solid was crystallized from ethanol to give 0.5 g. (33%) of **5** which melted at 189-190°; uv: λ max (*p*H 1) 347 nm (17,400) 277 nm (3,100) 239 nm (3,500); λ max (*p*H 11) 332 nm (15,700) 300 nm infl.

Anal. Calcd. for $C_{10}H_{14}N_2O_6S$: C, 41.38; H, 4.86; N, 9.65. Found: C, 41.49; H, 4.67; N, 9.54.

5-Methoxycytidine (**6**).

A solution of 5.0 g. of 5-methoxy-4-thio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrimidin-2-one (**4**) in 150 ml. of methanol (saturated with ammonia at 0°) was heated in a small sealed tube immersed in an oil bath which was maintained at 93° for 21 hours. The sealed tube was cooled, vented, the methanol solution treated with carbon, filtered and evaporated. The resulting solid was triturated with chloroform and was then crystallized by the addition of ethanol. The solid was filtered and dried to give 2.04 g. (84%) of **6** which melted at 192-194°; uv: λ max (*p*H 1) 302 nm (10,200) λ max (*p*H 11) 289 nm (7,500). The pmr gave a sharp singlet at 3.42 δ which integrated for 2 protons (water of hydration).

Anal. Calcd. for $C_{10}H_{15}N_3O_6 \cdot H_2O$: C, 41.23; H, 5.88; N,

14.43. Found: C, 40.91; H, 5.67; N, 14.18.

2,2′-Anhydro-1-β-D-arabinofuranosyl-5-methoxyuracil (**7**).

A mixture of 7.8 g. of 5-methoxy-1-β-D-ribofuranosyluracil (**3**), 7.9 g. of diphenylcarbonate and 0.1 g. of sodium bicarbonate in 25 ml. of dimethylformamide was stirred and heated at 150° for 30 minutes. Solution was obtained in *ca.* 3 minutes. The reaction was cooled to 80° and then poured carefully into 1 l. of ether with vigorous stirring. The stirring was continued for ½ hour and the solid was then removed by filtration, heated in 150 ml. of methanol, cooled, filtered and dried to give 5.65 g. of **7**. Evaporation of the mother liquor provided an additional 0.4 g. of **7** with the overall yield being 83%. Crystallization from methanol provided material which melted at 251-253°; uv: λ max (*p*H 1) 263 nm (8,700) λ max (*p*H 11) 266 nm (8,500).

Anal. Calcd. for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C, 46.66; H, 4.45; N, 10.93.

1-β-D-Arabinofuranosyl-5-methoxyuracil (**8**).

A stirred mixture of 5.65 g. of 2,2′-anhydro-1-β-D-arabinofuranosyl-5-methoxyuracil (**7**) in 200 ml. of 0.1 *N* sodium hydroxide was heated on the steam bath for 1 hour. Tlc on silica gel showed that starting material still remained so enough sodium hydroxide to make the solution 0.125 *M* was added and heating was continued 15 minutes. The solution was cooled, neutralized with Dowex 50 (H^+), filtered and evaporated to dryness. The solid was triturated with 110 ml. of boiling methanol, cooled, filtered and dried to give 4.76 g. (79%) of **8**. The solid was recrystallized from methanol to give colorless fine needles which melted at 235-237°; uv: λ max (*p*H 1) 279 nm (8,800) λ max (*p*H 11) 276 nm (7,300).

Anal. Calcd. for $C_{10}H_{14}N_2O_7$: C, 43.80; H, 5.15; N, 10.22. Found: C, 43.82; H, 5.36; N, 10.34.

1-β-D-Arabinofuranosyl-5-methoxycytosine (**12**).

A solution of 4.76 g. of 1-β-D-arabinofuranosyl-5-methoxyuracil (**8**) in 20 ml. of dry pyridine and 20 ml. of acetic anhydride was left at room temperature for 2 hours. Tlc on silica gel showed the reaction to be complete. The solution was evaporated to dryness leaving 6.0 g. of foam. The resulting foam resisted all attempts at crystallization. The foam was therefore treated directly in pyridine (75 ml.) using phosphorus pentasulfide (6.0 g.). After the reaction had been refluxed for 5 hours the volume was concentrated to *ca.* ½ and then poured into 2 l. of water at 60°. The water was cooled and extracted with chloroform (5 x 50 ml.). The chloroform was extracted with 3 *N* sulfuric acid (2 x 25 ml., to remove pyridine), saturated sodium chloride solution (2 x 15 ml.) and then dried over sodium sulfate. Evaporation of the chloroform left *ca.* 6.0 g. of yellow foam which resisted crystallization (17).

A 2.45 g. portion of foam (**10**) was dissolved in 20 ml. of methanol, heated to 45° and ammonia bubbled through for 1 week. Methanol was replenished as necessary and the reaction was followed by uv shift. After 1 week the solution was evaporated to dryness, the solid triturated with chloroform and the residue crystallized from methanol to give 1.22 g. (49% for 2 steps) of **12** which melted at 207-208.5°; uv: λ max (*p*H 1) 302 nm (9,800); λ max (*p*H 11) 288 nm (7,600).

Anal. Calcd. for $C_{10}H_{15}N_3O_6$: C, 43.95; H, 5.53; N, 15.38. Found: C, 43.95; H, 5.90; N, 15.03.

1-β-D-Arabinofuranosyl-5-methoxy-4-thiopyrimidin-2-one (**11**).

A 1.9 g. fraction of foam was dissolved in 100 ml. of methanol and 100 mg. of sodium methoxide was added. After 4 days at room temperature tlc indicated the reaction was complete. Dowex 50 (H^+) was used to neutralize the base, the mixture was filtered and the methanol evaporated to dryness. Ethanol (25 ml.) was added and the resulting product was filtered and dried to give 0.80 g. (35% for 2 steps) of **11**. Recrystallization from ethanol gave yellow-orange material which melted at 213-213.5°; uv: λ max (*p*H 1) 348 nm (20,600) 277 nm (4,000) 240 nm (4,400); λ max (*p*H 11) 332 nm (18,700) 299 nm infl.

Anal. Calcd. for $C_{10}H_{14}N_2O_6S$: C, 41.38; H, 4.86; N, 9.65. Found: C, 41.61; H, 5.05; N, 9.39.

1-(2-Deoxy-β-D-*erythro*pentofuranosyl)-5-methoxyuracil (**14**).

A solution of 0.90 g. of 2-deoxy-3,5-di-*O*-*p*-tolyl-D-*erythro*-pentofuranosyl chloride and 0.80 g. of 2,4-*bis*(trimethylsilyl)-5-methoxyuracil (**1**) in 25 ml. of dry acetonitrile was left at room temperature for 64 hours. The solution was evaporated to dryness, the solid boiled in 50 ml. of ethanol and then 5 ml. of water added. The solution was cooled, evaporated and the solid triturated with chloroform. The chloroform was evaporated and the resulting solid was then crystallized from 10 ml. of methanol. The solid melted over the range 160-172°. Attempts at separation were unsuccessful.

The solid (1.0 g.) was dissolved in 50 ml. of methanol and 50 mg. of sodium methoxide was added. Solution of the solid did not occur in 6 hours so 10 ml. of tetrahydrofuran was added. The tetrahydrofuran was removed by distillation, enough methanol added to maintain the 50 ml. volume and the solution was then refluxed for 10 hours. The reaction was cooled, adjusted to neutrality with Dowex 50 (H^+), filtered and evaporated to dryness. The solid was triturated with chloroform and the remaining solid crystallized from methanol (4 ml.). The product was filtered and dried to give 0.16 g. (31%) of **14** which melted at 204.5-205°. A second crystallization from methanol-ethanol gave an analytical sample which melted at 207.5-208°; uv: λ max (*p*H 1) 278 nm (8,300); λ max (*p*H 11) 276 nm (6,700).

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.47; N, 10.85. Found: C, 46.60; H, 5.11; N, 10.64.

REFERENCES

(1) E. C. Hermann, Jr., *Proc. Soc. Exptl. Biol. Med.*, **107**, 142 (1961).

(2) W. H. Prusoff, *Pharmacol. Rev.*, **19**, 209 (1967) and references therein.

(3) M. M. Nemes and M. R. Hilleman, *Proc. Soc. Exptl. Biol. Med.*, **119**, 515 (1965).

(4) J. Shapira, L. Lowden and R. Hale, *J. Bacteriol.*, **83**, 919 (1962).

(5a) T. J. Bardos, M. P. Kotick and C. Szantay, *Tetrahedron Letters*, 1759 (1966). (b) M. P. Kotick, C. Szantay and T. J. Bardos, *J. Org. Chem.*, **34**, 3806 (1969). (c) G. L. Szekeres and T. J. Bardos, *J. Med. Chem.*, **13**, 708 (1970).

(6) A. Bloch, M. H. Fleysher, R. Thedford, R. F. Mane and R. H. Hall, *ibid.*, **9**, 886 (1966).

(7) S. H. Schwartz, T. J. Bardos, G. H. Burgess and E. Klein, *J. Medicine*, **1**, 174 (1970).

(8a) Kindly supplied by Dr. T. J. Bardos and the anti-viral test run by Dr. R. W. Sidwell at ICN Nucleic Acid Research Institute. 5-IdU had a V.R. of 1.0-1.8 in the same system. (b) R. W. Sidwell and J. H. Huffman, *Appl. Microbiol.*, **22**, 797 (1971).

(9) J. H. Chesterfield, J. F. W. McOmie and M. S. Tute, *J. Chem. Soc.*, 4590 (1960).

(10a) L. Birkofer, A. R. Ritter and H. P. Kulthan, *Angew. Chem.*, 75, 209 (1963). (b) E. Wittenberg, *Z. Chem.*, 4, 303 (1964).

(11) M. G. Stout and R. K. Robins, *J. Org. Chem.*, 33, 1219 (1968).

(12) Z. Budějínský, J. Přikryl and E. Svátek, *Collect. Czech. Chem. Commun.*, 29, 2980 (1964).

(13) M. W. Winkley and R. K. Robins, *J. Org. Chem.*, 33, 2822 (1968).

(14) A. Hampton and A. W. Nichol, *Biochemistry*, 5, 2076 (1966).

(15) M. Hoffer, *Chem. Ber.*, 93, 2777 (1960).

(16) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, 87, 4934 (1965).

(17) The foam was separated into two fractions by chromatography using 150 g. of silica gel (Baker 3405) in a column 4.0 x 35 cm. The initial wash with 2 l. of chloroform removed some trace impurities and the two products were then eluted with 2.5 l. of chloroform-ethyl acetate (4:1). The two bands were easily visible on the column. The solvent containing the faster band was evaporated to give 2.45 g. of foam and the slower band gave 1.9 g. of foam. Neither fraction was crystallized. Tlc against 4-thio-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)pyrimidin-2-one and uv analysis indicated that the 2.45 g. portion was 5-methoxy-4-thio-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)pyrimidin-2-one (10) and the 1.9 g. portion was a diacetyl derivative of 11. It appears that partial deacetylation of the product had taken place during work-up.