stirring 1 hr longer, 10–20 ml of H_2O was added and the MnO_2 was removed by filtration. The filtrate was made acidic by addition of 2–3 ml of 3 *N* HCl, and the resulting suspension was heated at 100° for 0.5 hr, cooled, and filtered. Extraction of the filtrate with Et_2O provided 48 mg of oil. Glpc indicated this to be about 80% *p*-chlorobenzaldehyde and 20% 4-methyl-5-nitrothiazole. The latter component was isolated by glpc in crystalline form, mp 48–50.5°. Its ir spectrum was identical with that of authentic 4-methyl-5-nitrothiazole (mp 52–53.5°), prepared by nitration of 4-methylthiazole,[22] and quite different from that of 2-methyl-5-nitrothiazole.

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Branched-Chain Sugar Nucleosides. V. Synthesis and Antiviral Properties of Several Branched-Chain Sugar Nucleosides

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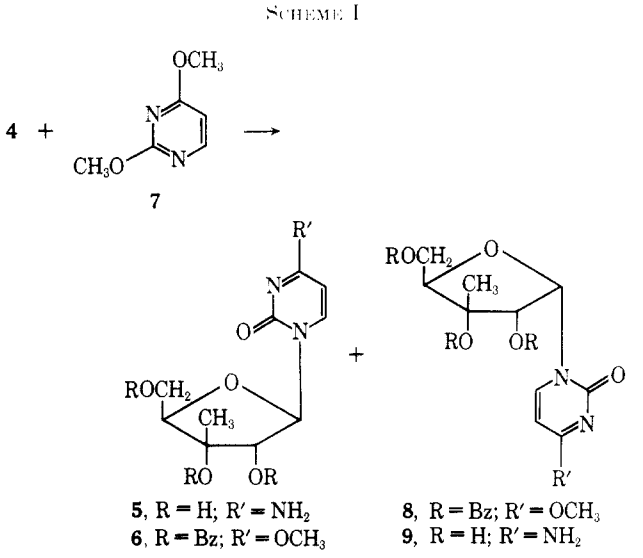
The synthesis of 3′-C-methylcytidine and its α-D anomer as well as 2′-C-methylcytidine, 2′-C-methyl-5-fluorocytidine, and 2′-C-methyl-5-fluorouridine *via* the Hilbert–Johnson reaction is described. In the synthesis of 2′-C-methylcytidine from N-acetylcytosinemercury a preponderance of the "O-glycoside" was formed. Biological testing indicates that 3′-C-methylcytidine as well as the previously synthesized 2′- and 3′-C-methyladenosines are effective antivaccinia agents in mice.

In earlier publications we described the synthesis of 2′-C-methyladenosine (**1**)[1a,b] and 3′-C-methyladenosine (**2**)[1c] from the novel branched-chain glycosyl halides 2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl chloride (**3**) and 2,3,5-tri-O-benzoyl-3-C-methyl-α- (and β-) D-ribofuranosyl bromide (**4**), respectively. We have now used the halides **3** and **4** in the synthesis of several related pyrimidine 2′- and 3′-C-methyl nucleosides. This paper describes the syntheses of these compounds. The effective antiviral activity shown by 2′-C-methyladenosine (**1**), 3′-C-methyladenosine (**2**), and 3′-C-methylcytidine (**5**), as evidenced by the protection they afford mice infected with neurovaccinia, is also reported. These branched-chain sugar nucleosides are representatives of a new class of synthetic antiviral agents.

For the synthesis of 3′-C-methylcytidine (**5**), 2,3,5-tri-O-benzoyl-3-C-methyl-D-ribofuranosyl bromide (**4**) was converted to 1-(2,3,5-tri-O-benzoyl-3-C-methyl-β-D-ribofuranosyl)-4-methoxy-2(1H)-pyrimidinone (**6**) by a Hilbert–Johnson[2] reaction with 2,4-dimethoxypyrimidine (**7**) (Scheme I). In addition to **6**, the α-D anomer **8** was isolated from the reaction mixture in a yield about one-tenth that of the β-D anomer **6**. Reaction of the pyrimidinones **6** and **8** with methanolic ammonia produced 3′-C-methylcytidine **5** and its α-D anomer **9**, respectively.

In contrast, the Hilbert–Johnson reaction between 2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl chloride (**3**) and 2,4-dimethoxypyrimidine (**7**) was very sluggish (Scheme II). Chromatography of the reaction products yielded the desired 1-(2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl-4-methoxy-2(1H)-pyrimidinone (**10**), but failed to indicate that any of the α-D anomer of **10** had been produced.[3] When **10** was heated in methanolic NH_3, 2′-C-methylcytidine (**14**) was obtained.

SCHEME I



In a similar manner, reaction of the glycosyl chloride **3** with 2,4-dimethoxy-5-fluoropyrimidine (**15**)[4] produced 1-(2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)-5-fluoro-4-methoxy-2(1H)-pyrimidinone (**16**), which

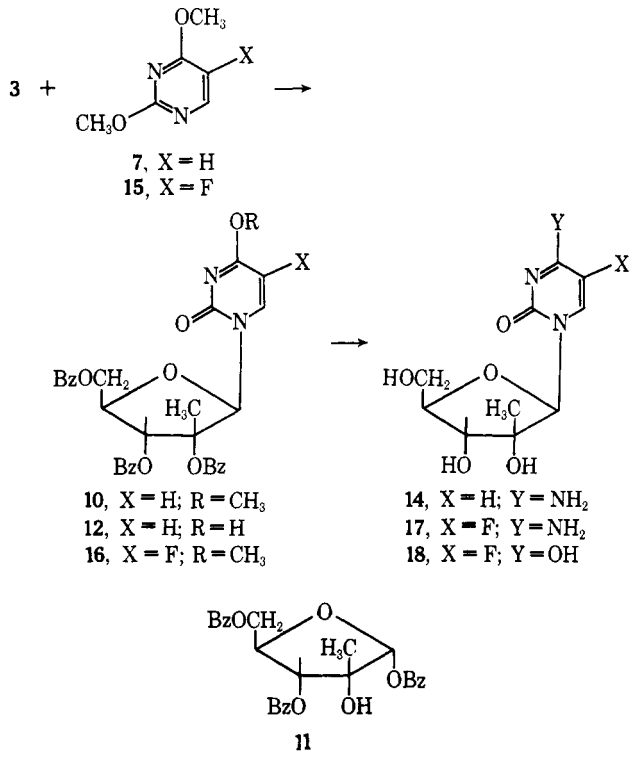
(1) (a) E. Walton, S. R. Jenkins, R. F. Nutt, M. Zimmerman, and F. W. Holly, *J. Amer. Chem. Soc.*, **88**, 4524 (1966); (b) S. R. Jenkins, B. Arison, and E. Walton, *J. Org. Chem.*, **33**, 1798 (1968); (c) R. F. Nutt, M. J. Dickinson, F. W. Holly, and E. Walton, *ibid.*, **33**, 2490 (1968).

(2) G. E. Hilbert and T. B. Johnson, *J. Amer. Chem. Soc.*, **52**, 2001 (1930).

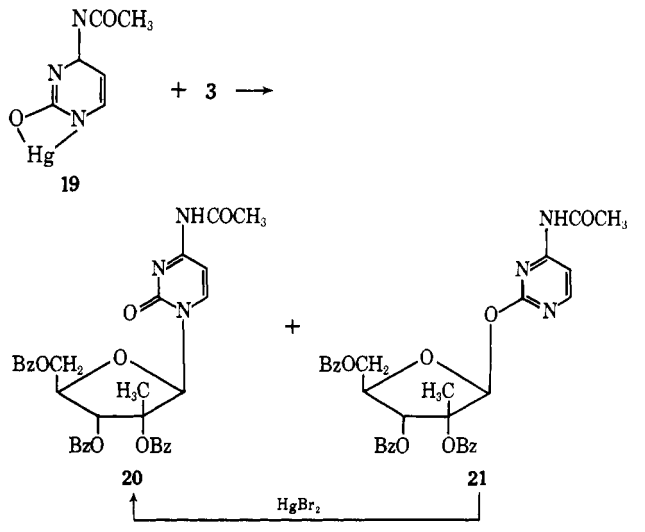
(3) T. J. Bardos, M. P. Kotick, and C. Czantay, *Tetrahedron Lett.*, 1759 (1966), have shown that in reactions of silated pyrimidines with D-glycosyl halides, high temperatures favor the β-D configuration, whereas at low temperatures the α-D product predominates. The isolation of only β-D products in the reaction of **3** with alkoxypyrimidines may be a result of the high reaction temperatures required.

(4) M. Prystas and F. Sorm, *Collect. Czech. Chem. Commun.*, **30**, 1900 (1965).

SCHEME II



SCHEME III



was subsequently converted into 5-fluoro-2′-C-methylcytidine (**17**) and 5-fluoro-2′-C-methyluridine (**18**).

2′-C-Methylcytidine was also prepared from **3** and N-acetylcytosinemercury (**19**). When **3** and N-acetylcytosinemercury reacted, 1-(2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)-4-acetamido-2(1H)-pyrimidinone (**20**) was formed, but in low yield; the major reaction product was 2-(2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyloxy)-4-acetamidopyrimidine (**21**)[5] (Scheme III). The ribofuranosyloxy derivative **21** was rearranged to **20** in refluxing xylene containing $HgBr_2$.[6] The rearrangement was slow and was accompanied by considerable decomposition with the formation of **11**[1b] and the yield of **20** was only 25%. Ammonolysis of **20** produced 2′-C-methylcytidine, identical with that obtained by the Hilbert–Johnson method.

Configurational Assignments.—The ORD curves of the products **5**, **14**, **17**, and **18** all showed positive Cotton effects, whereas that of **9** showed a negative Cotton effect which is in keeping with the configurational assignments.[7] The "*trans* rule"[8] predicts that the 2′-C-methylcytidine obtained from the reaction of **3** with N-acetylcytosinemercury would be of the β-D configuration. That it was identical with the product from the Hilbert–Johnson reaction supports the proposal that all of the products, except **9**, obtained from **3** *via* Hilbert–Johnson reactions are also of the β-D configuration.

Biological Activity.—The role of nucleosides in the suppression of DNA virus replication has been studied extensively, both in the *in vitro*[9] and *in vivo*[10] host systems. The studies reported herein are concerned with the activity of branched-chain sugar nucleosides in the suppression of dermal lesions in the vaccinia-infected mouse. The use of the tail vein assay system is advantageous in that it is highly sensitive and compares favorably in reliability to severe testing procedures for systemic manifestation of neurovaccinia infections. The test system here reported results in a self-limiting disease offering opportunity to observe the onset, progress, and ultimate regression of the disease process.

Data relating the antivaccinia effect of the test and reference compounds are shown in Table I. The relationship of drug concentration to range of lesion within a given test group with the resultant median lesion count suggests a dose-dependency response in the case of the active compounds.

2′-C-Methyladenosine and N-methylisatin 3-thiosemicarbazone[11] at the 2.0-mg level were comparable in

(5) Although the formation of "O-glycosides" in the synthesis of pyrimidine nucleosides by the mercury method is not uncommon, the formation of the O derivative (**21**) in the present case was unexpected. Previously N-acetylcytosinemercury, with a base to mercury ratio of 1:1, has yielded N-glycosyl derivatives exclusively in reaction with glycosyl halides; see, for example, M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Amer. Chem. Soc.*, **81**, 4112 (1959); J. J. Fox, N. C. Yung, I. Wempen, and M. Hoffer, *ibid.*, **83**, 4066 (1961); J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *ibid.*, **79**, 5060 (1957); H. M. Kissman and M. J. Weiss, *ibid.*, **80**, 2595 (1958); C. L. Stevens and K. Nagarajan, *J. Med. Pharm. Chem.*, **5**, 1124 (1962); C. L. Stevens and P. Blumbergs, *J. Org. Chem.*, **30**, 2723 (1965). During the course of this work H. G. Garg and T. L. V. Ulbricht, *J. Chem. Soc., C*, 51 (1967), reported the first observation of the formation of an O-glycoside in the reaction of N-acetylcytosinemercury with 3,4,6-tri-O-acetyl-2-deoxy-2-(2′,4′-dinitroanilino)-α-D-glucopyranosyl bromide. They suggested that the formation of the O-glycoside may be related to the lowered reactivity of their glycosyl halide. However, the reaction of **3** with N-acetylcytosinemercury was rapid (30 min) compared to the slow reaction (5 hr) noted by Garg and Ulbricht. The recovery of O-glycoside in the present case is more likely due to the more restrictive steric interaction of the pyrimidine moiety with the 2′-C-methyl group in **20** than in **21**.

(6) (a) G. Wagner and H. Pischel, *Naturwissenschaften*, **48**, 454 (1961); (b) T. Ukita, H. Hayatsu, and Y. Tomita, *Chem. Pharm. Bull.* (Tokyo), **11**, 1068 (1963).

(7) T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, *Tetrahedron Lett.*, 695 (1964).

(8) B. R. Baker, Ciba Foundation Symposium, Chemistry and Biology of Purines, Little, Brown and Co., Boston, Mass., 1957, p 120.

(9) (a) E. C. Hermann, *Proc. Soc. Exp. Biol. Med.*, **107**, 142 (1961); (b) N. P. Salzman, A. J. Shatkin, and E. D. Sedring, *Ann. N. Y. Acad. Sci.*, **130**, 240 (1965).

(10) (a) H. D. Kauffman, *Proc. Soc. Exp. Biol. Med.*, **109**, 251 (1962); (b) P. Calabresi, R. W. McCollum, and A. D. Welch, *Nature*, **197**, 763 (1963).

(11) Marboran®, methisazone. D. J. Bauer and P. W. Sadler, *Brit. J Pharmacol. Chemotherapy*, **15**, 101 (1960).

TABLE I

COMPARATIVE ANTIVACCINIA EFFECT OF BRANCHED-CHAIN SUGAR NUCLEOSIDES

Agent	Total dose, mg	Range of lesions per group	Median count	Lesion index[a]
2′-C-Methyladenosine	2.0	0, 0, 0, 2, 2, 6, 7, 10, 15, >15	2	7.5
	1.0	1, 3, 5, 7, 7, 8, 10, 10, 12, >15	7	2.1
	0.5	0, 3, 3, 10, 10, 10, 15, 15, 17, >15	10	1.5
3′-C-Methyladenosine	2.0	0, 0, 0, 0, 0, 0, 0, 0, 3, 4, 0	0	>15
	1.0	0, 0, 0, 0, 1, 2, 6, 8, 10	1	15
	0.5	0, 0, 0, 1, 4, 4, 5, 8, 10	4	3.75
Adenosine	2.0	3, 4, 5, 6, 10, 12, 12, 15	10	1.5
	1.0	>15 entire group	>15	<1
2′-C-Methylcytidine	2.0	3, 12, 10, >15, >15, etc.	>15	<1
	1.0	8, 10, 15, >15, >15, etc.	>15	<1
	0.5	1, 4, 15, >15, >15, etc.	>15	<1
3′-C-Methylcytidine	2.0	0 entire group	0	>15
	1.0	0, 0, 0, 0, 0, 1, 1, 2, 5, 8	0	>15
	0.5	2, 3, 5, 6, 10, 12, 12, 12, 15, 15	10	1.5
Cytidine	2.0	10, 15, >15, etc.	>15	<1
	1.0	4, 7, 10, 10, 12, >15, etc.	12	1.25
N-Methylisatin 3-thiosemicarbazone	2.0	1, 2, 2, 2, 3, 3, 3, 3, 8, 12	3	5
Saline controls	0.5 ml	>15 entire group	>15	<1

[a] Lesion index = median count of control animals/median count of test animals.

effect with lesion indices of 7.5 and 5, respectively. Decrease in the total dosage resulted in an increase in the number of dermal lesions.

There was a measurable increase in activity demonstrated by the compounds having branching at the 3′ position. Both 3′-C-methyladenosine and 3′-C-methylcytidine were highly active at the 2.0- and 1.0-mg levels. 3′-C-Methyladenosine continued to show a high level of activity with a dosage as low as 0.5 mg, whereas 3′-C-methylcytidine had a diminishing activity at this dose level and compared in activity to 2′-C-methyladenosine.

No studies were conducted to ascertain the mechanism of action of these nucleosides in the suppression of vaccinia virus.

Experimental Section[12]

1-(2,3,5-Tri-O-benzoyl-3-C-methyl-β-D-ribofuranosyl)-4-methoxy-2(1H)-pyrimidinone (6) and Its α-D Anomer (8).—A mixture of 4.08 mmoles of **4**[1c] and 1.3 g (9.27 mmoles) of **7**[2] in 75 ml of CH_2Cl_2 was kept at 25° for 5 days. Tlc on alumina in $CHCl_3$–C_6H_6 (3:1) showed zones at R_f 0.2 (**8**), 0.6 (**6**), 0.8 (**7**). About 50 ml of CH_2Cl_2 was added and the solution was extracted with cold 5% HCl and cold 5% $KHCO_3$, dried ($MgSO_4$), and concentrated. The residual solid was chromatographed on 50 g of alumina (acid-washed, Merck) eluting with first C_6H_6–$CHCl_3$ (4:1), then C_6H_6–$CHCl_3$ (1:4), and finally with $CHCl_3$. Concentration of selected (tlc) fractions gave, after crystallization (C_6H_6–petroleum ether (bp 30–60°), 1.07 g (45%) of **6**: mp 84–90°; $[\alpha]D$ −76° (*c* 1, $CHCl_3$); uv max (CH_3OH), 230 mμ (log ε 43.4), 275 (9.4), 280 (8.6). *Anal.* ($C_{32}H_{28}N_2O_9$) C, H, N.

Later fractions gave the α anomer (**8**) which was recrystallized (C_6H_6–petroleum ether); yield 120 mg (5%); mp 206–209°; $[\alpha]D$ −180° (*c* 0.5, $CHCl_3$); uv max (CH_3OH), 229 mμ (log ε 38.0), 275 (9.3), 280 (8.6). *Anal.* C, H, N.

3′-C-Methylcytidine (5).—A mixture of 500 mg (0.856 mmole) of **6** in 7.5 ml of MeOH saturated with NH_3 at 0° was heated (sealed tube) for 20 hr at 100°. The solution was concentrated and the residue, in 50 ml of H_2O, was washed with three portions of Et_2O to remove benzamide. Concentration of the H_2O layer gave crystals which when recrystallized (MeOH) gave 201 mg (92%) of **5**: mp 235–238°; $[\alpha]D$ +4° (*c* 0.5, H_2O); φ +190° (350 mμ), +2180 (300) +3300 pk (290), 0 (273) (*c* 0.0547, H_2O); nmr (D_2O), τ 4.02 (d, C-1′ H, $J_{1',2'}$ = 7.5 Hz):[13] uv max (H_2O pH 1), 213 mμ (log ε 10.6), 279 (12.9); (pH 7), 233 (8.1), 271 (8.9); (pH 13), 230 (8.2), 271 (8.9). *Anal.* ($C_{10}H_{15}N_3O_5$) C, H, N.

1-(3-C-Methyl-α-D-ribofuranosyl)cytosine (9).—By the method used to prepare **5**, 50 mg of **8** was converted into **9**. Recrystallization (MeOH) yielded 20 mg (92%): mp 250–258°; $[\phi]$ +17,800° (230 mμ), +20,200 pk (245), 0 (272), −18,800 tr (287) (*c* 0.043, H_2O). *Anal.* ($C_{10}H_{15}N_3O_5$) C, H, N.

1-(2,3,5-Tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)-4-methoxy-2(1H)-pyrimidinone (10).—A solution of 5.4 g (10 mmoles) of **3**[1b] in 50 ml of dry PhMe was added to 2.8 g (20 mmoles) of **7** in 50 ml of dry PhMe and the solution was refluxed for 5 days. The solvent was removed and the residue (8.7 g) in 200 ml of Et_2O was washed (5% HCl, saturated $NaHCO_3$, H_2O). The Et_2O was removed and the residue (5.35 g) was chromatographed on 250 g of silica gel in C_6H_6–EtOAc (19:1). Early fractions yielded 2.2 g of unidentified products derived from **3**, followed by 1.0 g of **11** and 1.85 g (32%) of **10** isolated as a noncrystalline glass: $[\alpha]D$ −21° (*c* 1, $CHCl_3$); R_f 0.22, tlc on silica in C_6H_6–EtOAc (9:1); uv max (EtOH), 229 mμ (log ε 42.8), 275 (9.1), 280 (8.3). *Anal.* ($C_{32}H_{28}N_2O_9$) C, H, N.

The column was stripped with EtOAc which yielded 550 mg of less mobile material which on tlc on silica gel in C_6H_6–EtOAc (4:1) showed zones at R_f 0.0, 0.1, 0.2, 0.3, and 0.4. Column chromatography in the same system gave fractions containing 90 mg of two-component material of R_f 0.2 and 0.1. This was rechromatographed in C_6H_6–EtOAc (1:1) and gave, after crystallization from 0.5 ml of C_6H_6, 35 mg of 1-(2,3,5-tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)uracil (**12**): mp 200–201°; $[\alpha]D$ −23° (*c* 1, $CHCl_3$); uv max (EtOH), 230 mμ (log ε 43.6), 255 (15.2); R_f 0.6, tlc on silica gel in C_6H_6–EtOAc (1:1). *Anal.* ($C_{31}H_{26}N_2O_9$) H, N; C: calcd, 65.26; found, 65.78.

Later fractions gave, after crystallization from 0.3 ml of benzene, 12 mg of 1-(2-O-acetyl-3,5-di-O-benzoyl-β-D-ribofuranosyl)-4-methoxy-2(1H)-pyrimidinone (**13**): uv max (EtOH), 229 mμ (log ε 31.0), 275 (8.7); nmr ($CDCl_3$), τ 3.38 (s, C-1′ H), 7.88 (s, 2′-$OCOCH_3$), 8.42 (s, 2′-CH_3). *Anal.* ($C_{27}H_{26}N_2O_9$) C, H, N.

(12) Where analyses are indicated only by the symbols of the elements, analytical results for those elements were within ±0.4% of the theoretical values. All melting points were determined on a micro hot stage and are corrected. Solvent concentrations were carried out at reduced pressure in a rotary evaporator. Except where noted the tlc zones were made visible with I_2 vapor. Fritted-glass Büchner funnels of medium porosity were used for column chromatographic separations. The silica gel (J. T. Baker, 100–200 mesh) packing had a height to diameter ratio of about 1:1. The nmr spectra were determined with a Varian Associates Model A-60 or, where noted, Model HA 100 spectrometer.

(13) The coupling constant, $J_{1',2'}$ = 7.5 Hz, indicates a rather large dihedral angle for $H_{1'}$–$H_{2'}$ which, by means of the same reasoning presented earlier[1c] for 3′-C-methyladenosine, suggests that the sugar moiety of **5** exists in a 3-*exo*-2-*endo* twist conformation (T_3^2). The resonance of the C-5 proton, which has a chemical shift almost the same as that of the C-1′ proton, was very broad and poorly resolved. A sharp doublet ($J_{5,6}$ = 7.5 Hz) for the C-5 proton was obtained by (1) heating the probe to 80° or (2) adding 1 drop of 0.1 *N* NaOD solution to the probe.

2′-C-Methylcytidine (14) from 10.—By the method used for the preparation of **5**, 1.0 g (1.7 mmoles) of **10** was converted into **14**. Recrystallization (MeOH) gave 394 mg (90%) of **14** as solvate, mp 243–245° (transition 140–170°). After being dried at 110° for several hours at reduced pressure **14** had mp 243–244°; $[\alpha]_D$ +132° (*c* 0.5, H_2O); ϕ +4000° (400 mμ), +19,200 pk (288), 0 (272), −21,800 tr (245) (*c* 0.051, H_2O). *Anal.* ($C_{10}H_{13}N_3O_5$) C, H, N.

1-(2,3,5-Tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)-5-fluoro-4-methoxy-2(1H)-pyrimidinone (16).—By the procedure used to synthesize **6**, 4.8 g (9.7 mmoles) of **3** and 3.5 g (22.2 mmoles) of **15**[3] were converted into **16**. After purification by chromatography on silica gel in C_6H_6–EtOAc (19:1) followed by recrystallization (C_6H_6–Et_2O), there was obtained 3.2 g (55%) of **16**, mp 157–159°, $[\alpha]_D$ −14° (*c* 1, $CHCl_3$). *Anal.* ($C_{32}H_{27}FN_2O_9$) C, H, F, N.

5-Fluoro-2′-C-methyluridine (18).—A suspension of 603 mg (1.0 mmole) of **16** in 20 ml of MeOH in 2 ml of H_2O and 170 mg (4.0 mmoles) of NaOH was refluxed 45 min and the solution was concentrated. The residue was dissolved in 20 ml of H_2O and Dowex 50X-4 (H^+) resin was added until the pH was 4. The resin and precipitated benzoic acid were removed and washed (H_2O) and the combined H_2O solutions were washed six times with Et_2O. The H_2O layer was concentrated and the residue was recrystallized (MeOH–Et_2O) twice to give 74 mg (27%) of **18**: mp 205–207°; $[\alpha]_D$ +90° (*c* 1, D_2O); ϕ +700° (400 mμ), +13,100 pk (288), 0 (274); nmr (D_2O), τ 4.03 (d C-1′ H, $J_{1'F}$ = 1.5 Hz). *Anal.* ($C_{10}H_{13}FN_2O_6$) C, H, F, N.

5-Fluoro-2′-C-methylcytidine (17).—By the procedure used in the synthesis of **5**, 80 mg (0.13 mmole) of **16** was converted into **17**. Recrystallization (MeOH–Et_2O) gave 24 mg (67%) of **17**: mp 247–249°; R_f 0.78, tlc on cellulose in H_2O; $[\phi]$ +1200° (400 mμ), +15,700 pk (302), 0 (281); nmr (D_2O), τ 4.10 (d, C-1′ H, $J_{1'F}$ = 1 Hz). *Anal.* ($C_{10}H_{14}FN_3O_5$) C, H, N.

1-(2,3,5-Tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)-4-acetamido-2(1H)-pyrimidinone (20) and 2-(2,3,5-Tri-O-benzoyl-2-C-methyl-D-ribofuranosyloxy)-4-acetamidopyrimidine (21).—2,3,5-Tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl chloride (**3**) (3.4 mmoles) in 75 ml of dry xylene was added to a suspension of 527 mg (2 mmoles) of **19** in 75 ml of dry xylene and the mixture was refluxed and stirred for 30 min. The reaction solution was concentrated to 35 ml, cooled, and treated with 175 ml of petroleum ether. The precipitated solid was removed, dissolved in 100 ml of $CHCl_3$, and washed with three 40-ml portions of 30% KI solution and two 40-ml portions of H_2O. The $CHCl_3$ solution was concentrated and the residue (1.2 g) was chromatographed on 40 g of silica gel in $CHCl_3$–EtOAc (1:1). The eluent was monitored by tlc on silica gel in the same solvent mixture. The first several column fractions contained two reaction products of R_f (tlc) 0.8 and 0.96. Later column fractions contained a product showing an R_f (tlc) of 0.23. These fractions were combined and concentrated to give 100 mg (13% based on **19**) of **2** as a glass: $[\alpha]_D$ −46° (*c* 0.86, $CHCl_3$); uv max (EtOH), 231 mμ (log ε 43.0), 273 infl (8.0), 283 (7.3), 300 (6.1). *Anal.* ($C_{33}H_{29}N_3O_9$) H, N; C: calcd, 64.80; found, 64.37.

The first products (R_f 0.8 and 0.96, 1.05 g) that were removed from the chromatographic column were rechromatographed on 40 g of silica gel in C_6H_6–EtOAc (19:1). Several fractions yielded 200 mg of by-products followed by fractions containing 600 mg of product which when crystallized twice from MeOH gave 400 mg (52%) of **21**: mp 99–100°; $[\alpha]_D$ +30.1° (*c* 1, $CHCl_3$); uv max (EtOH), 230 mμ (log ε 49.5), 274 (14.5). *Anal.* ($C_{33}H_{29}N_3O_9$) C, H, N.

1-(2,3,5-Tri-O-benzoyl-2-C-methyl-β-D-ribofuranosyl)-4-acetamido-2(1H)-pyrimidinone (20) from 2-(2,3,5-Tri-O-benzoyl-2-C-methyl-D-ribofuranosyloxy)-4-acetamidopyrimidine (21).—A solution of 100 mg (0.16 mmole) of **21** in 20 ml of dry xylene containing 180 mg (0.5 mmole) of $HgBr_2$ was refluxed for 4 hr, filtered, and concentrated. The residue was added to 20 ml of $CHCl_3$ and filtered, and the $CHCl_3$ solution was washed with three 15-ml portions of 30% KI and three 15-ml portions of H_2O. Concentration of the $CHCl_3$ layer gave 80.5 mg of residual glass which was chromatographed on silica gel in C_6H_6–EtOAc (1:1). After removal of **11** (R_f 0.8) fractions containing **20** were obtained. The yield of **20**, having properties identical with that prepared above, was 25 mg (25%).

2′-C-Methylcytidine (14) from 20.—By the method used to prepare **5**, 47 mg (0.08 mmole) of **20** was converted into 17 mg (80%) of **14** with properties identical with those of **14** prepared from **10**.

Biological Testing. Virus.—The WR strain of vaccinia virus was obtained from the American Type Culture Collection and maintained in this laboratory as part of the virus seed stock inventory. The stock pool used in these studies was the 25th mouse brain passage, stored at −80° as a 10% mouse brain suspension and had a mouse brain titer of $10^{6.3}LD_{50}$ per 0.03 ml. The appropriate dilution of virus used for intravenous inoculation was so standardized that discrete tail lesions appeared at 5 days but had no lethal effect on the drug-treated or placebo mice. Virus dilutions were prepared with nutrient broth.

Mice.—Random-bred male albino mice (IRC strain) weighing 9–11 g as obtained from the Merck Sharp and Dohme mouse breeding colony were used throughout these studies.

Compounds and Treatment Regimen.—The compounds used in the antiviral studies included the branched-chain sugar nucleosides (**1**, **2**, **6**, and **14**) described in this and earlier[1] publications. The compounds were dissolved in nutrient broth and diluted to contain 2.0, 1.0, and 0.5 mg/0.5-ml dose or approximately 200, 100, and 50 mg/kg, respectively. The respective compound dosage was administered to groups of ten mice each by the intraperitoneal route 3 hr prior to virus challenge. A single postinfection dose of compound was administered 18 hr later. A suspension of the reference compound, N-methylisatin 3-thiosemicarbazone (**13**) was prepared and administered as described above.

Virus Inoculation.—The appropriate virus dilution contained in 0.2 ml was administered intravenously. To facilitate this procedure, the tail veins were dilated by placing the mice in a thermostatically controlled warming box for approximately 10 min prior to inoculation.

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