appropriate equation.[29] The values of ΔS^*, as reported in Table II, were averaged from those obtained by this equation at all the temperatures used for each substrate. The probable errors in the rate constants lie between 2 and 5%, the probable errors in E_{act} between 0.5 and 1.2 kcal, and the probable errors in ΔS^* between 1.5 and 3 eu.

Registry No.—*N*-(2-methyl-4-nitrophenyl)piperidine, 51248-16-9; 2-chloro-5-nitrotoluene, 13290-74-9; piperidine, 110-89-4.

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Nucleic Acid Related Compounds. 12. The Facile and High-Yield Stannous Chloride Catalyzed Monomethylation of the Cis-Glycol System of Nucleosides by Diazomethane[1]

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Stannous chloride has been found to catalyze monomethylation of the cis-glycol system of nucleosides by diazomethane. With compounds having no acidic proton on the base, such as adenosine and cytidine, quantitative monomethylation occurs rapidly. Purine nucleosides gave a mixture of 2′-*O*-methyl and 3′-*O*-methyl isomers with the 3′ product in somewhat greater proportion. Pyrimidine nucleosides gave a mixture in which the 2′-*O*-methyl isomer predominates significantly. It has been found that even nucleosides which contain an acidic proton on the base can be monomethylated on the sugar by raising the concentration of catalyst and slowly adding a dilute solution of diazomethane. The 2′-*O*- and 3′-*O*-methyl ethers of adenosine, 6-chloropurine riboside, tubercidin, formycin, guanosine, cytidine, 4-methoxy-1-β-D-ribofuranosyl-2-pyrimidinone, uridine, and pseudouridine have been directly prepared in this manner. Thus, 2′-*O*-methyl nucleosides isolated from ribonucleic acids (as well as their 3′-*O*-methyl isomers) are now readily available by this route. Mass spectroscopy is shown to be a convenient and useful tool for the investigation of the isomeric structures.

Although 2′-*O*-methyl ethers of the "major" nucleosides are ubiquitous "minor components" of various ribonucleic acids,[3] routes to their syntheses have often been rather laborious. Several indirect approaches have been employed involving multistep procedures, especially for compounds containing an acidic hydrogen on the heterocyclic base. No generally applicable direct route has been available previously.

Ribonucleic acids (RNA's) containing 2′-*O*-methylnucleosides give rise to di- (and higher) nucleotide fragments containing these molecules upon digestion with sodium hydroxide,[4] since intermediate 2′,3′,5′-*O*-phospho triester formation necessary for cleavage to 2′(3′)-mononucleotides is blocked by the 2′-methyl ether function. Certain 2′-*O*-methyl-5′-nucleotides formed upon venom diesterase hydrolysis of RNA's were reported to be resistant to snake venom 5′-nucleotidase.[4,5] Hydrolase and phosphorylase enzymes which catalyze cleavage of the sugar–base linkage of nucleosides were found to have no effect on 2′-*O*-methyluridine[5] or the synthetic 3′-*O*-methyluridine.[6] Various 2′-*O*-methyl polynucleotide systems have been prepared and studied[7] in order to evaluate effects of the 2′-hydroxyl group in "normal" polynucleotides, effects on helix complementarity, hydrophobic contributions, etc. The possibility of employing these altered enzymatic and chemical properties in the design of nucleoside "drugs" with enhanced or protected (against catabolism and degradation) biological properties has been discussed.[8-12]

Broom and Robins[13] first exploited diazomethane for the selective sugar methylation of nucleosides by treating a hot aqueous solution of adenosine with diazomethane in glyme (1,2-dimethoxyethane). They reported isolation of 2′-*O*-methyladenosine in some 40% yield. Gin and Dekker[14] reported similar results but also isolated 3′-*O*-methyladenosine in 11% yield plus other minor methylated products. Reese and coworkers[15] reported analogous re-

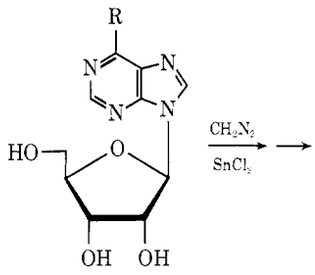
sults, with 2′-*O*- and 3′-*O*-methyladenosine being isolated in about 40% combined yield and a ratio of ~3:1, respectively. Cytidine was methylated by this procedure to give 2′-*O*-methylcytidine in less than 20% yield plus some 3′-*O*-methylcytidine after a rather extensive work-up procedure.[15] Deamination of these derivatives gave an indirect synthesis of 2′-*O*- and 3′-*O*-methyluridine in low overall yields. Methylation of dityrituridine derivatives and separation of 2′-*O*- and 3′-*O*-methyluridine from the corresponding *N*,*O*-dimethyl products has been reported.[6] A recent multistep sugar synthesis and base coupling procedure gave 2′-*O*-methyluridine and its N^3-coupled isomer.[16] Methylation of 2-amino-6-chloro-9-β-D-ribofuranosylpurine and conversion of the heterocyclic moiety has been reported as a route to 2′-*O*-methylguanosine.[17] A multistep sugar synthesis, base coupling, and subsequent heterocyclic conversion was used to synthesize 3′-*O*-methylguanosine.[18] Recently, Shugar and coworkers have investigated the direct alkylation of cytosine nucleosides with dialkyl sulfates in strong aqueous alkali.[10-12] A similar approach was employed by Ts'o to effect 2′-O-alkylation of adenosine and cytidine 3′,5′-cyclic phosphates.[7c] This procedure leads to mixtures of alkylated products, and the separation of all possible combinations of even sugar alkylation products is an involved task. Certain isomers predominate, however, and apparently have fortuitous separation properties.[12] The yield of any one product is low to moderate unless exhaustive alkylation of suitably blocked precursors is employed.[7c,12]

We wish to describe a very facile and high yield procedure for the monomethylation of the cis-glycol system of nucleosides[19] using diazomethane with stannous chloride dihydrate as catalyst in methanolic (and other organic solvents) solution.[20] Interestingly, boron trifluoride etherate is ineffective in this system, although other metal salts give pronounced effects.[21]

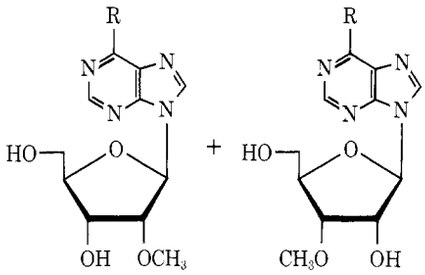
Treatment of a suspension of adenosine (**1a**) in methanol containing 10^{-3} *M* stannous chloride dihydrate with a solution of diazomethane in glyme gave quantitative reaction with accompanying solubility of products. Evaporation of volatiles and chromatography of the residue on Dowex 1-X2 (OH^-)[22] gave 61% of 3′-*O*-methyladenosine (**3a**) and 38% of 2′-*O*-methyladenosine (**2a**) as pure crystalline solids.

Analogous reaction of 6-chloro-9-β-D-ribofuranosylpurine (**1b**) gave 6-chloro-9-(2-*O*-methyl-β-D-ribofuranosyl)purine (**2b**, 30%) and 6-chloro-9-(3-*O*-methyl-β-D-ribofuranosyl)-purine (**3b**, 38%) as analytically pure crystalline solids after preparative thin layer chromatographic (tlc) separation. The noncatalyzed methylation of **1b** with diazomethane has been reported, but **2b** was not purified or characterized and no investigation of the 3′-*O*-methyl isomer (**3b**) was mentioned.[17] Quantitative conversion of samples of **2b** and **3b** to the corresponding adenosine compounds **2a** and **3a** using liquid ammonia[23] confirmed the structure assignments. Treatment of **2b** and **3b** with hydrosulfide gave 9-(2-*O*-methyl-β-D-ribofuranosyl)purine-6-thione[17] (**2c**) and 9-(3-*O*-methyl-β-D-ribofuranosyl)purine-6-thione[18] (**3c**), respectively. Paterson and coworkers[24] have used *p*-nitrobenzylthio-substituted nucleosides as inhibitors of nucleoside transport across cell walls. Sulfur alkylation of **2c** and **3c** with *p*-nitrobenzyl bromide proceeded readily to give 6-*p*-nitrobenzylthio-9-(2-*O*-methyl-β-D-ribofuranosyl)purine (**2d**) and 6-*p*-nitrobenzylthio-9-(3-*O*-methyl-β-D-ribofuranosyl)purine (**3d**) for transport binding studies.

The nucleoside antibiotic tubercidin (**4a**) exerts strong biological inhibition in various systems and several molecular mechanisms have been investigated.[25] Catalyzed methylation gave 2′-*O*-methyltubercidin [4-amino-7-(2-*O*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine, **5a**, 53%] and 3′-*O*-methyltubercidin [4-amino-7-(3-*O*-methyl-

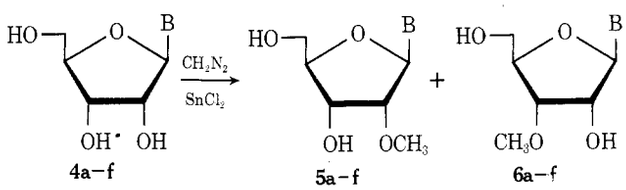


1a, R = NH_2
b, R = Cl

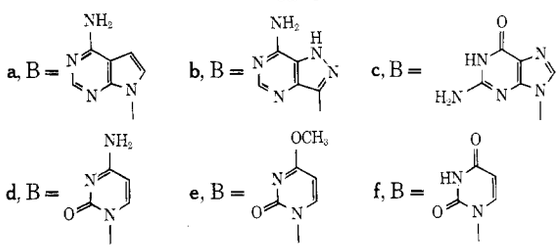


2a–d **3a–d**

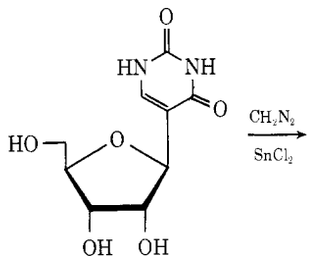
a, R = NH_2
b, R = Cl
c, R = SH
d, R = $SCH_2C_6H_4NO_2$-*p*



4a–f **5a–f** **6a–f**



a, B = **b**, B = **c**, B =
d, B = **e**, B = **f**, B =



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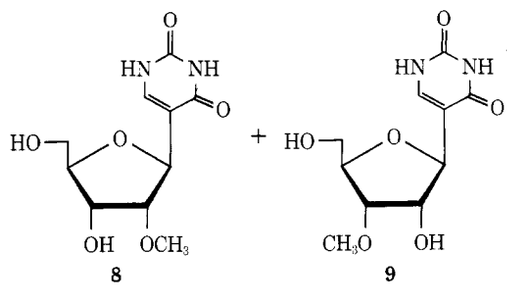


8 **9**

β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine, **6a**, 39%] after separation on the Dekker[22] column. Nmr and mass spectral (see later discussion) trends follow the adenosine case. However, in order to obtain conclusive proof of the isomer structures, each compound was acylated with trifluoroacetic anhydride and subjected to nmr double resonance analysis. The 2′-*O*-methyl nucleoside gives a 3′,5′-bis(*O*-trifluoroacetyl) derivative and the 3′-*O*-methyl isomer the 2′,5′-bis(trifluoroacetate). The primary alcoholic protons ($H_{5',5''}$) are shifted downfield by over 1δ in both cases confirming a free 5′-OH. The $H_{2'}$ of the 2′-*O*-methyl derivative is shifted only slightly upon trifluoroacetylation, whereas the $H_{3'}$ is shifted downfield ~0.9δ. Similarly, $H_{2'}$ of the 3′-*O*-methyl derivative is shifted ~0.9δ downfield, while $H_{3'}$ remains essentially unchanged. Irradiation of the nonshifted ($H_{2'}$ or $H_{3'}$) peak and observation of the collapse (2′-*O*-methyl isomer) of the anomeric ($H_{1'}$) proton doublet to a singlet or its ($H_{1'}$) invariance (3′-*O*-methyl isomer), respectively, verified the structures as assigned.

Formycin (**4b**) is another nucleoside antibiotic of considerable current interest.[26] Owing to its "glycosidic" linkage at C_3, an acidic proton (pK_a = 9.5)[27] is present on N_1 of the pyrazole moiety. In the presence of stannous chloride, however, the sugar was selectively monomethylated in good yield with only minor quantities of additionally methylated products detected. The products were separated by preparative tlc to give 2′-*O*-methylformycin [7-amino-3-(2-*O*-methyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine, **5b**] and 3′-*O*-methylformycin [7-amino-3-(3-*O*-methyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine, **6b**]. Nmr and mass spectral trends were analogous to those of the adenosine isomers and, again, trifluoroacetylation and proton double resonance experiments confirmed the isomer structure assignments.

Guanosine (**4c**) is readily methylated at N_7 by diazomethane;[28] additional methylation of the guanine base during 2′-deoxyguanosine treatment with diazomethane has been reported.[29] Indeed, attempted sugar methylation of 2-amino-6-benzyloxy-9-β-D-ribofuranosylpurine using diazomethane in aqueous glyme was reported to give N_7-methylation and imidazole ring opening.[17] However, careful treatment of a solution of guanosine (**4c**) and stannous chloride dihydrate in *N,N*-dimethylformamide gave 2′-*O*-methylguanosine (**5c**) and 3′-*O*-methylguanosine (**6c**) in ~50% combined yield. Separation of **5c** and **6c** from other methylated products was effected using silica gel column chromatography. The isomers, **5c** and **6c**, were cleanly resolved (3′ isomer again eluting first[30]) on a Dowex 1-X2 (Cl^-) column using an ammonium chloride–ammonium hydroxide buffer (pH 10.0). This anion exchange system[30] also separated 2′-*O*-methyl- and 3′-*O*-methylinosines (which can be prepared directly under analogous conditions but in lower yield) and would appear to be a useful procedure to consider for separation problems involving closely related isomers with an acidic ring proton in this general pH range.

Cytidine (**4d**) gave 2′-*O*-methylcytidine (**5d**, 74%) and 3′-*O*-methylcytidine (**6d**, 15%) as analytically pure crystalline products after chromatographic resolution,[22] in contrast with low yields reported in the absence of catalyst.[15]

Earlier studies in our laboratory[8] on the uncatalyzed methylation of 4-methoxy-1-β-D-ribofuranosyl-2-pyrimidinone (**4e**) resulted in isolation of 4-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**5e**) in only 37% yield. Formation of 4-methoxy-1-(3-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**6e**) and the 2′,3′-di-*O*-methyl nucleoside were noted, but **6e** was not isolated or characterized. The present catalyzed methylation of **4e** gave **5e** (74%) and **6e** (15%) as pure crystalline products. Thus, these useful intermediates for the preparation of 4-substituted 2-pyrimidinone nucleosides[8] are now readily available.

Treatment of a methanolic solution of uridine (**4f**) with diazomethane in glyme gives high yields of 3-*N*-methyluridine analogously to reported findings.[31] This result may be anticipated, since uridine has an acidic proton (pK_a = 9.2)[32] on N_3. However, treatment of **4f** and stannous chloride dihydrate in methanol with diazomethane in glyme gave 2′-*O*-methyluridine (**5f**, 58%) and 3′-*O*-methyluridine (**6f**, 28%) after preparative tlc separation. These yields and ease of direct reaction again stand in marked contrast with previous routes to 2′-*O*-methyluridine.[6,12,15,16,44]

The isolation of minute quantities of 2′-*O*-methylpseudouridine (**8**) from tRNA has been reported,[33] but no synthetic sample has been available previously for comparison. Careful treatment of pseudouridine (**7**) gave an ~80% combined crude yield of 2′-*O*-methylpseudouridine [5-(2-*O*-methyl-β-D-ribofuranosyl)uracil, **8**] and 3′-*O*-methylpseudouridine [5-(3-*O*-methyl-β-D-ribofuranosyl)-uracil, **9**]. Silica gel column chromatography gave partial resolution of the two isomers, and pure samples of **8** (27%) and **9** (10%) were obtained. Conditions were not investigated for the optimization of these procedures owing to the cost of **7**. The isomeric structures of **8** and **9** were again indicated by nmr and mass spectral trends and verified by trifluoracetylation and nmr decoupling experiments. It is amazing that the catalytic enhancement is sufficient to allow sugar hydroxyl (pK_a > 13)[34] methylation to occur preferentially in the presence of the two acidic (pK_a ~ 8.9)[34] uracil ring protons (N_1 H and N_3 H) of ψ(**7**). The advantages of such a direct procedure are accentuated in the preparation of this natural product **8**, since transformation of other naturally occurring nucleosides to the pseudouridine system is precluded and *C*-glycoside coupling is not presently inviting.[35]

An examination of ^1H nmr data (see Table I) reveals that the anomeric proton ($H_{1'}$) is uniformly at lower field in the 2′-*O*-methyl isomer than in the 3′-*O*-methyl isomer as previously noted by Reese.[15] It is interesting that the $-OCH_3$ resonance is at lower field for the 3′ isomer in the purine and purine-like (tubercidin and formycin) compounds whereas this trend is uniformly reversed (2′–OCH_3 at lower field than 3′–OCH_3) in the pyrimidine series, including the pseudouridines.

Selected mass spectral fragmentation patterns (see Table II) show several trends. As previously noted,[36,37] the purine ring seems better able to stabilize charge localization in the base relative to the pyrimidine ring. This effect is seen in the lower abundance of the molecular ion (M) [and various other ions containing the heterocyclic base (B) plus a sugar fragment] in the pyrimidine compounds. The pseudouridine isomers (**8** and **9**) had extremely weak M peaks and the M + 1 intensity was always about fourfold greater. The uridine products **5f** and **6f** also had significant M + 1 peaks. Pseudouridine[38] gave an M + 1 peak of higher intensity than the M ion in several spectra obtained on our equipment. This was not noted in previously reported spectra[39,40] and its cause is unknown.

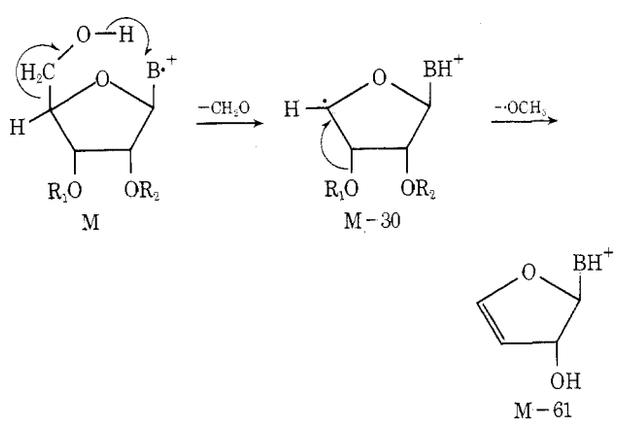
A peak at M − 15, presumably demethylation of the molecular ion, was noted in the purine and purine-like compounds except **2d**. This fragmentation was essentially absent from all the pyrimidine derivatives and was of lower intensity in the 2′ isomers except **5b**.

A peak at M − 30 is observed in all these nucleosides. This ion was proposed[36] to arise by loss of the hydroxymethyl function (with 5′-OH hydrogen transfer to base) as formaldehyde to give the M − 30 ion shown.

Table I
Characteristic ^{1}H Nmr Bands of the Methylated Nucleosides[a]

Compd	Solvent[b]	2′-OCH$_3$[c]	3′-OCH$_3$[c]	H$_{1'}$[d]	Base protons
2a	A	3.49		6.05 (5.6)	8.04 (s, H$_2$), 8.22 (s, H$_8$)
3a	A		3.60	6.01 (5.8)	8.05 (s, H$_2$), 8.25 (s, H$_8$)
2b	A	3.37		6.09 (4.8)	8.54 (s), 8.58 (s); H$_2$, H$_8$
3b	A		3.48	6.02 (5.1)	8.53 (s), 8.59 (s); H$_2$, H$_8$
2c	B	3.28		5.95 (5.0)	8.16 (s), 8.51 (s); H$_2$, H$_8$
3c	B		3.36	5.83 (5.4)	8.19 (s), 8.52 (s); H$_2$, H$_8$
2d	B	3.32		6.10 (5.0)	8.75 (s), 8.80 (s); H$_2$, H$_8$
3d	B		3.37	5.96 (5.5)	8.72 (s), 8.74 (s); H$_2$, H$_8$
5a	A	3.24		6.02 (6.3)	6.36 (d, H$_5$), 7.11 (d, H$_6$) (J_{5-6} = 4.0 Hz), 7.87 (s, H$_2$)
6a	A		3.42	5.94 (6.2)	6.36 (d, H$_5$), 7.13 (d, H$_6$) (J_{5-6} = 3.9 Hz), 7.85 (s, H$_2$)
5b[e]	A	3.39		5.28 (6.5)	8.38 (s, H$_5$)
6b[e]	A		3.45	5.15 (6.3)	8.31 (s, H$_5$)
5c	B	3.28		5.77 (5.8)	7.93 (s, H$_5$)
6c	B		3.34	5.63 (6.0)	7.88 (s, H$_5$)
5d	A	3.55		5.98 (3.3)	6.05 (d, H$_5$), 7.88 (d, H$_6$) (J_{5-6} = 7.5 Hz)
6d	A		3.48	5.89 (3.9)	6.04 (d, H$_5$), 7.84 (d, H$_6$) (J_{5-6} = 7.5 Hz)
5e	A	3.58		6.02 (2.7)	6.27 (d, H$_5$), 8.25 (d, H$_6$) (J_{5-6} = 7.5 Hz)
6e	A		3.47	5.93 (2.5)	6.26 (d, H$_5$), 8.22 (d, H$_6$) (J_{5-6} = 7.3 Hz)
5f	A	3.53		5.98 (3.6)	5.90 (d, H$_5$), 7.92 (d, H$_6$) (J_{5-6} = 8.3 Hz)
6f	A		3.45	5.87 (4.8)	5.89 (d, H$_5$), 7.88 (d, H$_6$) (J_{5-6} = 8.2 Hz)
8	A	3.46		4.73 (4.8)	7.64 (s, H$_6$)
9	A		3.39	4.68 (5.3)	7.58 (s, H$_6$)

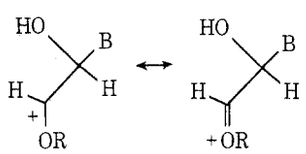
[a] Chemical shifts given in δ (parts per million from external TMS for D_2O solutions and internal TMS for DMSO-d_6). [b] A = D_2O; B = DMSO-d_6. [c] Sharp singlets. [d] Doublets; number in parentheses is $J_{1'-2'}$. [e] Spectrum taken on the monohydrochloride salt.



This fragmentation was observed to be uniformly more intense for the 2′ isomers (R_1 = H; R_2 = CH_3), although differences are often insignificant. An ion at M − 61 (not observed in the pyrimidine spectra) is seen to be much more intense in the 3′ isomers of the purine and purine-like compounds. Loss of a methoxyl radical from the M − 30 ion to produce the dihydrofuran derivative (M − 61) shown has been suggested.[41]

Loss of a methoxyl radical[37] from the molecular ion is presumed to give the M − 31 peak seen in all of these spectra. This is a low-intensity ion, especially in the pyrimidine and guanine nucleosides. A peak at M − 32 (essentially absent in the purine and purine-like compounds) appears characteristic of 2′-O-methylation in pyrimidine nucleosides. In certain other pyrimidine nucleoside pairs (to be published), only the 2′-*O*-methyl isomer has a significant M − 32 peak also. The 1′,2′ elimination of methanol would give a dihydrofuran derivative. Such extended conjugation could be advantageous in stabilizing the presumed less favorable charge localization[37] in the pyrimidine bases.

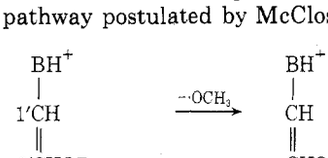
The B + 74 and B + 60 peaks correspond to ions suggested previously.[36,37] Although originally proposed to



B + 74 (R = CH_3); B + 60 (R = H)

arise *via* (and require) labile hydrogen transfer from a 3′ heteroatom,[36] an analogous peak was observed in permethylated nucleosides.[37] As seen in Table II this fragmentation is *not* uniformly diagnostic of the isomeric pairs.

The B + 58 and B + 44 ions are presumed to arise primarily by the pathway postulated by McCloskey.[36] These



B + 58 (R = CH_3)
B + 44 (R = H)
B + 43

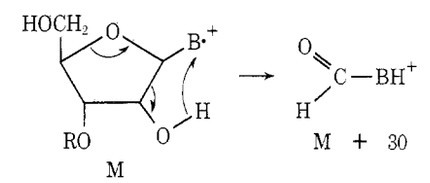
ions are seen to be uniformly diagnostic for all the isomeric pairs. The B + 58 peak is significantly more intense than the B + 44 peak for the 2′-*O*-methyl products, while the B + 44 peak is greater than the B + 58 peak for the 3′-*O*-methyl isomers (2′-hydroxyl free). Thus, inspection of the abundances of these two peaks in the spectrum of a compound specifies which isomer it is. It may be noted

Table II. Selected Mass Spectral Ions, *m/e* (Relative Intensity)

Compd	Temp, °C	M	M − 15	M − 30	M − 31	M − 32	M − 61	B + 74	B + 60	B + 58	B + 44	B + 30	B + 2H	B + H	*m/e* 146	*m/e* 87	Other ions
2a	180	281 (6.7)	266 (0.4)	251 (24)	250 (6.6)		220 (1.4)	208 (2.9)	194 (0.6)	192 (73)	178 (2.6)	164 (51)	136 (96)	135 (100)	(54)	(18)	
3a	170	281 (4.3)	266 (13)	251 (11)	250 (2.8)		220 (23)	208 (0.1)	194 (1.4)	192 (2.2)	178 (26)	164 (92)	136 (100)	135 (96)	(0.8)	(14)	
2b	145	300 (1.3)	285 (0.4)	270 (41)	269 (4.8)		239 (2.8)	227 (0.9)	213 (24)	211 (74)	197 (3.2)	183 (27)	155 (69)	154 (8.5)	(100)	(63)	
3b	135	300 (4.4)	285 (5.3)	270 (15)	269 (1.4)		239 (17)	227 (0.8)	213 (6.7)	211 (4.0)	197 (26)	183 (95)	155 (100)	154 (7.0)	(3.8)	(25)	
2c	160	298 (3.7)	283 (1.1)	268 (11)	267 (3.0)		237 (0.7)	225 (0.5)	211 (1.6)	209 (25)	195 (3.0)	181 (13)	153 (50)	152 (100)	(40)	(31)	
3c	200	298 (3.0)	283 (3.1)	268 (3.6)	267 (0.5)		237 (5.0)		211 (0.5)	209 (1.1)	195 (8.6)	181 (33)	153 (59)	152 (100)	(1.3)	(28)	
2d	150	433 (22)		403 (2.1)	402 (0.3)		372 (0.2)		346 (0.4)	344 (7.1)		316 (1.3)	288 (11)	287 (50)	(9.1)	(25)	136 (100, $C_7H_6NO_2$)
3d	190	433 (32)	418 (0.6)	403 (0.3)	402 (1.3)		372 (3.5)			344 (0.2)	330 (4.3)	316 (5.0)	288 (22)	287 (100)	(0.9)	(36)	136 (26, $C_7H_6NO_2$)
5a	200	280 (8.5)	265 (0.3)	250 (4.6)	249 (2.5)		219 (0.5)	207 (1.3)	193 (0.2)	191 (23)	177 (1.1)	163 (17)	135 (31)	134 (100)	(12)	(16)	
6a	200	280 (4.8)	265 (2.9)	250 (2.1)	249 (1.2)		219 (5.6)		193 (0.3)	191 (1.6)	177 (9.8)	163 (55)	135 (39)	134 (100)	(0.3)	(7.3)	
5b	190	281 (2.2)	266 (4.8)	251 (7.1)	250 (3.9)		220 (1.7)	208 (1.1)	194 (1.1)	192 (100)	178 (22)	164 (74)	136 (4.9)	135 (11)	(1.5)	(3.6)	
6b	220	281 (5.2)	266 (1.8)	251 (1.7)	250 (2.0)		220 (3.2)	208 (1.3)	194 (1.0)	192 (16)	178 (34)	164 (100)	136 (2.3)	135 (3.9)	(1.2)	(4.5)	
5c	190	297 (6.8)	282 (0.2)	267 (2.2)	266 (0.4)		236 (0.3)	256 (0.3)	242 (0.3)	208 (14)	194 (0.7)	180 (2.4)	152 (19)	151 (100)	(7.2)	(54)	
6c	240	297 (7.3)	282 (0.8)	267 (0.7)	266 (0.7)		236 (5.1)	256 (0.4)		208 (0.1)	194 (6.6)	180 (9.0)	152 (26)	151 (100)	(1.9)	(19)	
5d	225	257 (0.7)		227 (2.7)	226 (1.4)	225 (1.8)		184 (1.6)	170 (0.1)	168 (11)	154 (1.9)	140 (20)	112 (100)	111 (15)	(42)	(33)	151 (74)
6d	200	257 (0.7)		227 (1.6)	226 (0.6)				170 (25)	168 (1.1)	154 (6.4)	140 (24)	112 (100)	111 (9.8)	(0.8)	(13)	151 (12)
5e	175	272 (0.5)		242 (1.5)	241 (0.6)	240 (2.5)		199 (1.8)	185 (4.5)	183 (9.5)	169 (3.1)	155 (13)	127 (100)	126 (4.2)	(24)	(69)	166 (47)
6e	180	272 (0.5)		242 (0.7)	241 (0.3)				185 (22)	183 (1.6)	169 (6.1)	155 (11)	127 (100)	126 (2.8)	(0.4)	(21)	166 (7.7)
5f	165	258 (0.8)		228 (0.8)	227 (0.8)	226 (3.5)		185 (2.1)	171 (0.1)	169 (2.1)	155 (0.3)	141 (1.9)	113 (13)	112 (2.7)	(24)	(100)	147 (21, sugar)
6f	225	258 (0.5)		228 (0.6)	227 (0.6)	226 (0.6)		185 (1.7)	171 (9.1)	169 (1.6)	155 (9.0)	141 (2.6)	113 (38)	112 (81)	(8.2)	(100)	147 (27, sugar)
8	200	258 (0.1)		228 (2.3)	227 (1.5)	226 (4.4)		185 (0.5)	171 (0.3)	169 (8.7)	155 (2.2)	141 (48)	113 (2.1)	112 (2.9)	(0.1)	(100)	259 (0.4, M + 1)
																	208 (8.8, furan derivative)
9	220	258 (0.2)		228 (1.2)	227 (0.9)	226 (1.9)		185 (0.4)	171 (13)	169 (5.9)	155 (12)	141 (82)	113 (0.3)	112 (0.6)	(0.2)	(100)	259 (0.5, M + 1)
																	208 (4.2, furan derivative)

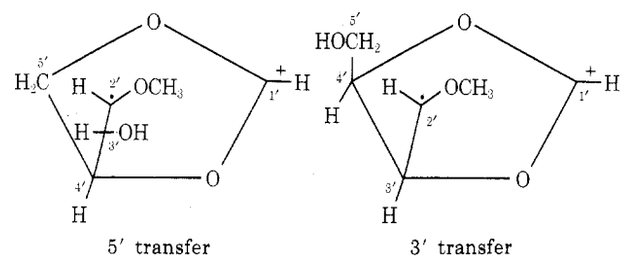
that rather minor differences in intensity in the same peak may exist when comparing a pair of isomers (*e.g.*, the B + 58 ion in **5f** and **6f**). Loss of the $O_{2'}$-methyl radical from the B + 58 ion to give a B + 43 ion was previously noted[36] with 2′-*O*-methyladenosine (**2a**). This step was suggested[36] to be unique to 2′-O-methylated compounds. However, the intensities of the B + 43 peak under our conditions were 4.1% for the 2′ isomer (**2a**) and 2.2% for the 3′ isomer (**3a**). This peak was absent or of less than 1% relative intensity in several 2′ isomers and was more intense in the 3′-*O*-methyluridine (**6f**) and pseudouridine (**9**) isomers.

The B + 30 ion, which contains $C_{1'}$, $H_{1'}$, and $O_{4'}$, arises by hydrogen transfer from sugar to base and $C_{4'}$–$O_{4'}$ and $C_{1'}$–$C_{2'}$ bond cleavages.[36] The suggested mechanism involving 2′-OH hydrogen transfer[36] is supported



by the greater intensity of this ion in the 3′-*O*-methyl isomers (free 2′-OH) of the purine and purine-like compounds only.

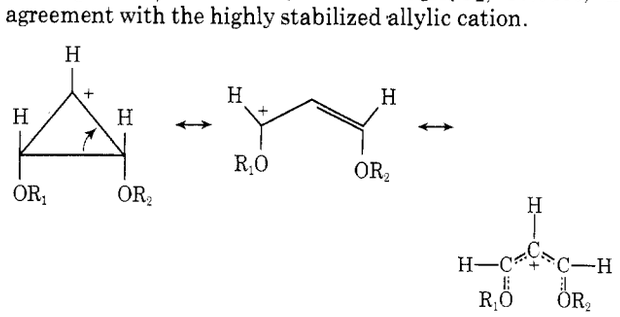
A significant peak at *m/e* 146 is observed for all 2′-*O*-methyl N-linked nucleosides, even constituting the mass spectral base peak (100% relative intensity) for **2b.** These



m/e 146

ions were postulated to arise from $C_{1'}$–$C_{2'}$ bond cleavage followed by 3′-OH or 5′-OH proton transfer to the base (B) with $C_{1'}$–base cleavage.[36] This peak is of low intensity in the 3′-*O*-methyl isomers and C-linked nucleosides, as predicted by the proposed mechanism.[36]

A significantly intense peak at *m/e* 87 was observed in the spectra of all these nucleosides, which was the mass spectral base peak for all four uridine (**5f** and **6f**) and pseudouridine (**8** and **9**) derivatives. Exact mass measurement gave *m/e* 87.0448 (calcd for $C_4H_7O_2$, 87.0446) in agreement with the highly stabilized allylic cation.



m/e 87

R_1 or R_2 = H, CH_3

An analogous species was observed in the mass spectra of permethylated nucleosides,[37] and an ion of *m/e* 73 (corresponding in mass to the above ion with $R_1 = R_2 = H$, $C_3H_5O_2$) was noted as a significant sugar fragmentation product of uridine.[40] Plausible mechanisms are difficult to

envision which would explain why the C-linked formycin compounds (**5b** and **6b**) give low-abundance *m/e* 87 peaks whereas the C-linked pseudouridines (**8** and **9**) have *m/e* 87 (100%). The high and sometimes nearly equal abundances of this ion in certain isomer pairs make consideration of fragmentations leading to a symmetrical intermediate (such as a cyclopropyl cation) involving the 2′ and 3′ carbons plus one additional CH group attractive.

Specific miscellaneous ions of note include the nitrobenzyl (or nitrotropylium) ion ($C_7H_6NO_2$) in the spectra of **2d** and **3d**, the strong sugar ion (*m/e* 147) in the uridine isomers, and the 5-(5-hydroxymethyl-2-furyl)uracil ion[40] (*m/e* 208) in **8** and **9**. The mass spectral base peak for 2′-*O*-methylformycin (**5b**) is the B + 58 ion (analogous to 2′-deoxyformycin[30]) while that for 3′-*O*-methylformycin (**6b**) is the B + 30 ion (as in 3′-deoxyformycin[30]). This is in accord with the preferred B + 58 fragmentation mechanism[36] and again[30] demonstrates that the B + 30 peak will *not necessarily* be the mass spectral base peak for a *C*-nucleoside, as had been suggested previously[42] and reemphasized very recently.[43]

This powerful catalytic system for monomethylation of nucleoside cis glycols makes direct access to the "minor component"[3] 2′-*O*-methylnucleosides (and their 3′ isomers) readily available. Mass spectral fragmentation trends are diagnostically useful for these isomers and provide results which support certain previously proposed mechanisms and preclude preferential or exclusive operation of others. Application of this general procedure to other products,[19] investigation of other catalysts,[21] and study of certain factors involved in the reaction will be reported separately.

Experimental Section

Melting points were determined on Fisher-Johns and Mel-Temp apparatus and are uncorrected. Uv spectra were recorded on Cary 14 or 15 spectrometers. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter using a 10-cm 1-ml microcell. Nmr spectra were recorded on Varian A-60, 56/60, and HA-100 spectrometers. Mass spectra were determined by the mass spectroscopy laboratory of this department on AEI MS-2 and MS-9 instruments at 70 eV using a direct probe for sample introduction at the specified temperature. Elemental analyses were determined by the microanalytical laboratory of this department and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Evaporations were effected using Büchler rotating evaporators under aspirator or mechanical oil pump vacuum at 40° or lower. Chromatography was effected on dry-packed columns of silica gel A (J. T. Baker 3405) or B (Mallinckrodt 2847). Preparative tlc plates were prepared using 110 g of Brinkmann PF 254 silica gel per 300 ml of water (slurry) or 300 g of Brinkmann PF 254 Type E alumina per 320 ml of water. These slurries produced four 20 × 40 cm plates of 0.8–1-mm thickness unless otherwise specified. Analytical tlc was run on Eastman silica gel sheets (13181).

Diazomethane Stock Solutions. Solution A. To an ice-cold mixture of 100 ml of 40% aqueous KOH and 150 ml of 1,2-dimethoxyethane (glyme) was slowly added 15 g of *N*-nitroso-*N*-methylurea with vigorous stirring. Stirring was continued for an additional 20 min at 0° and the phases were allowed to separate. The upper organic layer was decanted and dried over KOH pellets. The dried solution was filtered before use. Standardization of the diazomethane solution using 0.2 *N* benzoic acid in glyme and back titration with 0.1 *N* NaOH gave an average value of 0.43 *N* CH_2N_2 in glyme.

Solution B[17] was prepared in the above manner using 25 g of *N*-nitroso-*N*-methylurea, 62 ml of 40% aqueous KOH, and 130 ml of glyme.

General Methylation Procedure. A round-bottom flask with a magnetic stir bar was charged with solvent, catalyst, and starting material. The mixture was stirred at room temperature (except guanosine at 50°). Diazomethane stock solution was added slowly and progress of the reaction was monitored by tlc. When all starting material disappeared or when further diazomethane failed to increase the yield of desired products, the reaction was terminated and the mixture was evaporated to dryness.

General Trifluoroacetylation Procedure. The compound was added to 2 ml of dried methylene chloride and 1 ml of trifluoroacetic anhydride and stirred for 3 days [20 hr in the case of 2′-*O*-methylpseudouridine (**8**)] at 5° while protected from moisture. The resulting clear solution was evaporated to dryness *in vacuo* and the residue was dissolved in dry solvent for nmr spectroscopy.

2′-*O*-Methyladenosine (2a) and 3′-*O*-Methyladenosine (3a). A suspension of 0.8 g (0.003 mol) of **1a** in 180 ml of a 10^{-3} *M* solution of $SnCl_2 \cdot 2H_2O$ in MeOH was treated with 44 ml of CH_2N_2 stock solution B by the general methylation procedure. The resulting residue was dissolved in 20 ml of EtOH–H_2O (2:1) and applied to a column (50 × 2 cm, 110 ml) of Dowex 1-X2 (OH^-) packed in the same solvent. The same solvent was used to develop the column and 10-ml fractions were collected. Fractions 21–30 were pooled and evaporated to give 0.30 g (38%) of crystalline **2a**, which was recrystallized from absolute EtOH to give product: mp 202–203.5°; $[\alpha]^{22}D$ − 58° (*c* 1, H_2O); uv (H_2O) max 259 nm (ε 14,500), min 226 nm (ε 2100) (see ref 13–15 and 17 for comparisons).

Anal. Calcd for $C_{11}H_{15}N_5O_4$: C, 47.00; H, 5.30; N, 24.90. Found: C, 47.16; H, 5.36; N, 24.61.

Fractions 41–70 were combined and evaporated to give 0.53 g (61%) of **3a**, mp 174–176°. This material was recrystallized from absolute EtOH to give **3a**: mp 177–178°; $[\alpha]^{22}D$ − 59° (*c* 1, H_2O); uv (H_2O) max 259 nm (ε 15,300), min 226 nm (ε 2200) (see ref 14, 15, and 18 for comparisons).

Anal. Found: C, 46.82; H, 5.22; N, 24.98.

6-Chloro-9-(2-*O*-methyl-β-D-ribofuranosyl)purine (2b) and 6-Chloro-9-(3-*O*-methyl-β-D-ribofuranosyl)purine (3b). A solution of 0.58 g (0.002 mol) of **1b** and 0.030 g (0.0001 mol) of $SnCl_2 \cdot 2H_2O$ in 120 ml of absolute EtOH was methylated with CH_2N_2 stock solution A by the general procedure. The residue obtained was dissolved in 10 ml of EtOH, and 4 g of silica gel (A) was added. This slurry was evaporated to dryness and the impregnated adsorbent was added to a column (2.2 cm diameter, 20 g) of the same silica gel. The column was developed with *i*-PrOH–$CHCl_3$ (4:96) and the major homogeneous fractions containing **2b** and **3b** were combined and evaporated to give a colorless, solid foam. This material was dissolved in absolute EtOH and applied to eight alumina preparative tlc plates (40 × 20 cm, 0.5 mm thick). The plates were air dried and were then developed in the lower layer of $CHCl_3$–Me_2CO–H_2O (3:2:1, to which 1 ml of EtOH per 100 ml of solution was added) for 6 hr. This procedure was repeated two or three times as needed to ensure clean separation of **2b** and **3b**.

The more rapidly migrating of the two major bands was scraped from the plates, powdered, packed into a 5-cm diameter column, and eluted with about 150 ml of absolute EtOH. This solution was evaporated and the resulting gum was dissolved in a minimum amount of absolute EtOH. Approximately 3 volumes of Skellysolve B was added and the solution was allowed to stand (at 4°). Over a period of time, 0.16 g of crystals, mp 151–153°, separated and an additional crop of 0.06 g, mp 150–153°, was obtained upon concentration of the mother liquor: total yield of **3b** 0.22 g (38%); $[\alpha]^{23}D$ −37.4° (*c* 0.96, MeOH); uv (H_2O) max 264 nm (ε 9000), min 227 nm (ε 2000).

Anal. Calcd for $C_{11}H_{13}ClN_4O_4$: C, 44.03; H, 4.37; Cl, 11.78; N, 18.66. Found: C, 44.27; H, 4.61; Cl, 11.74; N, 18.47.

The slower moving major band was treated in the same manner to provide 0.17 g (30%) of crystalline **2b**: mp 144–146°; $[\alpha]^{23}D$ −29.3° (*c* 0.99, MeOH); uv (H_2O) max 263 nm (ε 9000), min 227 nm (ε 2200).

Anal. Found: C, 43.81; H, 4.65; Cl, 11.74; N, 18.89.

9-(2-*O*-Methyl-β-D-ribofuranosyl)purine-6-thione[17] **(2c).** A stirred solution of 0.15 g (0.0005 mol) of **2b** in 5 ml of MeOH was treated with 1.5 ml of 1 *N* NaSH in MeOH and the reaction mixture was gently refluxed for 15 min. After cooling to room temperature, the solution was evaporated to dryness and the 0.18 g of residue obtained was dissolved in about 2 ml of hot H_2O. The pH was adjusted to 4.5 by dropwise addition of HOAc and 0.13 g (88%) of **2c** was obtained by filtration and drying at 0.1 mm. Crystalline **2c** had mp 248–249° dec; $[\alpha]^{25}D$ −56.6° (*c* 0.88, 0.1 *N* NaOH); uv (MeOH) max 226, 323 nm (ε 8100, 24,000), min 256 nm (ε 260) [lit.[17] mp >200° dec; $[\alpha]^{22}D$ −53.5° (*c* 0.42, 0.1 *N* NaOH)].

Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.29; H, 4.73; N, 18.78; S, 10.75. Found: C, 44.34, H, 4.92; N, 18.80; S, 10.52.

9-(3-*O*-Methyl-β-D-ribofuranosyl)purine-6-thione[18] **(3c).** A 0.15 g (0.0005 mol) portion of **3b** was treated exactly as in the conversion of **2b** to **2c** above to give 0.13 g (87%) of crystalline **3c**:

mp 215–218° dec; $[\alpha]^{25}D$ −77.6° (*c* 0.84, 0.1 *N* NaOH); uv (MeOH) max 226, 323 nm (ε 9100, 22,000), min 256 nm (ε 620) [lit.[18] mp 200–201° dec; $[\alpha]^{23}D$ −77° (*c* 1.0, 0.1 *N* NaOH)].

Anal. Calcd for $C_{11}H_{14}N_4O_4S$: C, 44.29; H, 4.73; N, 18.78; S, 10.75. Found: C, 44.12; H, 4.80; N, 18.89; S, 10.66.

6-*p*-Nitrobenzylthio-9-(2-*O*-methyl-β-D-ribofuranosyl)purine (2d). To a stirred solution of 0.175 g (0.00058 mol) of **2c** and 0.11 g of anhydrous Na_2CO_3 in 5 ml of dry DMF was slowly added a solution of 0.13 g (0.0006 mol) of α-bromo-*p*-nitrotoluene (*p*-nitrobenzyl bromide) in 3 ml of glyme. Stirring was continued for 2 hr at room temperature, the mixture was filtered, the filtrate was evaporated to dryness, and the residue was coevaporated with EtOH and then 5 ml of xylene. The resulting foam was dissolved in MeOH and applied to a silica gel preparative tlc plate (20 × 20 cm) which was developed in MeOH–$CHCl_3$ (10:90). The major band was scraped from the plate, extracted with MeOH, filtered, and evaporated to give an oil which was crystallized from EtOAc–cyclohexane. The analytically pure sample of **2d** (0.145 g, 57%) after drying at 0.1 mm had mp 125.5–128°; $[\alpha]^{25}D$ −38.9° (*c* 1.03, DMF); uv (MeOH) max 284 nm (ε 26,900), min 242 nm (ε 8400); uv (0.1 *N* HCl in MeOH) max 291 nm (ε 23,300), min 243 nm (ε 8700); uv (0.1 *N* NaOH in MeOH) max 284 nm (ε 26,400), min 243 nm (ε 8300).

Anal. Calcd for $C_{18}H_{19}N_5O_6S$: C, 49.87; H, 4.41; N, 16.15; S, 7.39. Found: C, 49.56; H, 4.65; N, 16.27; S, 7.47.

6-*p*-Nitrobenzylthio-9-(3-*O*-methyl-β-D-ribofuranosyl)purine (3d). A 0.18-g (0.0006 mol) portion of **3c** was treated as in the conversion of **2c** to **2d.** The resulting oil was crystallized from MeOH–cyclohexane plus a few drops of EtOAc to give 0.15 g (58%) of **3d:** mp 173–174°; $[\alpha]^{25}D$ −45.4° (*c* 1.0, DMF); uv (MeOH) max 284 nm (ε 27,000), min 240 nm (ε 5400); uv (0.1 *N* HCl in MeOH) max 292 nm (ε 22,700), min 240 nm (ε 5700); uv (0.1 *N* NaOH in MeOH) max 284 nm (ε 25,600), min 243 nm (ε 8000).

Anal. Calcd for $C_{18}H_{19}N_5O_6S$: C, 49.87; H, 4.41; N, 16.15; S, 7.39. Found: C, 49.83; H, 4.51; N, 15.99; S, 7.34.

4-Amino-7-(2-*O*-Methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (2′-*O*-Methyltubercidin, 5a) and 4-Amino-7-(3-*O*-methyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (3′-*O*-Methyltubercidin, 6a). A solution of 0.8 g (0.003 mol) of **4a** in 200 ml of a 10^{-3} *M* solution of $SnCl_2 \cdot 2H_2O$ in MeOH was treated with about 45 ml of CH_2N_2 stock solution B according to the general methylation procedure. The residue from evaporation was dissolved in 5 ml of EtOH–H_2O (2:1) and applied to a column (75 × 3 cm) of Dowex 1-X2 (OH^-) resin packed in and developed with the same EtOH–H_2O (2:1) solvent mixture. Fractions (6 ml) 21–45 contained a small amount of unidentified material. Fractions 131–200 were combined and evaporated to give a colorless, solid foam (0.45 g, 53%) which did not crystallize under several conditions. A solution of 0.1 g of this material in 10 ml of absolute EtOH was treated with 10 ml of 0.05 *N* HCl in absolute EtOH and evaporated to dryness. The residue was coevaporated twice with absolute EtOH and then crystallized from this solvent to give 0.1 g (90%) of **5a** monohydrochloride: mp 222–224°; $[\alpha]^{26}D$ −44° (*c* 1, H_2O); uv (H_2O) max 270 nm (ε 12,700), min 239 nm (ε 2400); uv (0.1 *N* HCl) max 270, 227 nm (ε 12,400, 25,300), min 244, 213 nm (ε 5000, 16,000); uv (0.1 *N* NaOH) max 270 nm (ε 13,200), min 240 nm (ε 4200).

Anal. Calcd for $C_{12}H_{17}ClN_4O_4$: C, 45.50; H, 5.37; Cl, 11.19; N, 17.69. Found: C, 45.78; H, 5.35; Cl, 11.04; N, 17.66.

A 0.02-g sample of **5a** was trifluoroacetylated with 2 ml of trifluoroacetic anhydride in 3 ml of methylene chloride at 0° for 0.5 hr, evaporated, and dried at 0.1 mm for 15 hr. The resulting solid foam gave nmr ($CDCl_3$, TMS internal) δ 3.36 (s, 1, 2′-OCH_3), 4.52 ("q," $J_{2'-1'} = J_{2'-3'} = 6$ Hz, 1, $H_{2'}$), 4.63 (m, 2, $H_{5',5''}$), 5.52 ("q," $J_{3'-2'} = 6$, $J_{3'-4'} = 4$ Hz, 1, $H_{3'}$), 6.18 (d, $J_{1'-2'} = 6$ Hz, 1, $H_{1'}$). Irradiation at δ 6.18 ($H_{1'}$) caused the "quartet" at δ 4.52 ($H_{2'}$) to collapse to a doublet ($J_{2'-3'} = 6$ Hz). Irradiation at δ 4.52 ($H_{2'}$) caused the "quartet" at δ 5.52 ($H_{3'}$) to collapse to a singlet (simultaneous irradiation of $H_{4'}$) and the doublet at δ 6.18 ($H_{1'}$) to collapse to a singlet.

Fractions 220–320 were combined and evaporated to dryness to give 0.33 g (39%) of solid, which was crystallized from H_2O to give the analytical sample of **6a:** mp 194–195°; $[\alpha]^{26}D$ −76.7° (*c* 1, H_2O); uv (H_2O) max 270 nm (ε 11,500), min 239 nm (ε 2900); uv (0.1 *N* HCl) max 270, 227 nm (ε 11,300, 23,900), min 244, 213 nm (ε 4900, 15,200); uv (0.1 *N* NaOH) max 270 nm (ε 11,700), min 240 nm (ε 4100).

Anal. Calcd for $C_{12}H_{16}N_4O_4$: C, 51.42; H, 5.75; N, 19.99. Found: C, 51.68; H, 5.74; N, 19.80.

This product (20 mg) was trifluoroacetylated exactly as **5a** above to give a product with nmr ($CDCl_3$, TMS internal) δ 3.42 (s, 1, 3′-OCH_3), 4.30 (m, 2, $H_{3'}$, $H_{4'}$), 4.48–4.81 (m, 2, $H_{5',5''}$), 5.83 ("q," $J_{2'-3'} = 5$, $J_{2'-1'} = 3$ Hz, 1, $H_{2'}$), 6.28 (d, $J_{1'-2'} = 3$ Hz, 1, $H_{1'}$). Careful irradiation at δ 6.28 ($H_{1'}$) caused collapse of the peak at δ 5.83 ($H_{2'}$) to a doublet ($J_{2'-3'} = 5$ Hz). Careful irradiation at δ 5.83 ($H_{2'}$) caused collapse of the peak at δ 6.28 ($H_{1'}$) to a singlet and sharpening of the band at δ 4.30 ($H_{3'}$). Irradiation at δ 4.30 ($H_{3'}$) had no effect on the δ 6.28 ($H_{1'}$) band but collapsed the δ 5.83 ($H_{2'}$) band to a doublet ($J_{2'-1'} = 3$ Hz).

7-Amino-3-(2-*O*-methyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (2′-*O*-Methylformycin, 5b) and 7-Amino-3-(3-*O*-methyl-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (3′-*O*-Methylformycin, 6b). A suspension of 0.40 g (0.0015 mol) of **4b** in 100 ml of 10^{-3} *M* $SnCl_2 \cdot 2H_2O$ solution was stirred while 15 ml of CH_2N_2 stock solution B was slowly added (further additions gave rise to more of the faster tlc migrating material, presumably dimethylated products). The residue obtained upon evaporation was treated with 5 ml of H_2O and filtered. The clear filtrate was applied to four silica gel preparative tlc plates (20 × 40 cm, 1 mm thick) which were thoroughly dried and then developed using $CHCl_3$–Me_2CO–EtOH–MeOH (20:10:1.5:1.5). The plates were dried and redeveloped three to four times in the same solvent system to effect splitting of the second fastest migrating band into two bands. The faster of these two bands was removed from the plates, extracted with MeOH, filtered, and evaporated to give 0.14 g (33%) of **5b** contaminated with a trace of **6b.** This solid did not crystallize readily and was dissolved in 3 ml of absolute EtOH and treated with 5 ml of 1 *N* HCl in absolute EtOH. This solution was evaporated to dryness and the residue was coevaporated twice and then crystallized using absolute EtOH to give 81 mg (17%) of **5b** monohydrochloride: mp 205–210° dec; $[\alpha]^{26}D$ −30.3° (*c* 0.98, H_2O); uv (H_2O) max 294 nm (ε 11,700), min 252 nm (ε 3300); uv (0.1 *N* HCl) max 296, 234 nm (ε 11,700; 10,000), min 269, 224 nm (ε 5600, 8900); uv (0.1 *N* NaOH) max 304, 234 nm (ε 8900, 17,800) min 272, 227 nm (ε 3700, 15,900).

Anal. Calcd for $C_{11}H_{16}ClN_5O_4$: C, 41.58; H, 5.08; Cl, 11.17; N, 22.04. Found: C, 41.93; H, 5.27; Cl, 11.15; N, 21.90.

A 20-mg sample of this product was trifluoroacetylated by the general procedure outlined to give nmr ($CDCl_3$, TMS internal) δ 3.35 (s, 3, 2′-OCH_3), 4.38–4.80 (m, 4, $H_{2'}$, $H_{4'}$, $H_{5',5''}$), 5.48 (d, $J_{1'-2'} = 7$ Hz, 1, $H_{1'}$), 5.60 ("q," $J_{3'-2'} = 5$, $J_{3'-4'} = 4$ Hz, 1, $H_{3'}$). Irradiation at δ 4.68 ($H_{2'}$) collapsed the δ 5.48 ($H_{1'}$) band to a singlet and the band at δ 5.60 ($H_{3'}$) to a broad singlet (simultaneous irradiation of $H_{4'}$ as well as $H_{2'}$). Careful irradiation at δ 5.48 ($H_{1'}$) raised a rough doublet at δ 4.7 ($H_{2'}$).

The slower of the two bands separated above by preparative tlc was treated exactly as **5b** to give 0.21 g (50%) of amorphous **6b.** This product was analogously converted into 0.2 g (85%) of crystalline **6b** monohydrochloride: mp 231–233°; $[\alpha]^{26}D$ −38.3° (*c* 1.1, H_2O); uv (H_2O) max 294 nm (ε 10,500), min 253 nm (ε 3000); uv (0.1 *N* HCl) max 295, 235 nm (ε 13,500, 8500) min 268, 224 nm (ε 4000, 6700); uv (0.1 *N* NaOH) max 305, 234 nm (ε 7800, 16,200), min 272, 226 nm (ε 3000, 14,000).

Anal. Calcd for $C_{11}H_{16}ClN_5O_4$: C, 41.58; H, 5.08; Cl, 11.17; N, 22.04. Found: C, 41.69; H, 4.95; Cl, 11.26; N, 21.99.

A 20-mg sample of this product was trifluoroacetylated by the general procedure to give nmr ($CDCl_3$, TMS internal) δ 3.42 (s, 3, 3′-OCH_3), 4.38 (m, 2, $H_{3'}$, $H_{4'}$), 4.48–4.78 (m, 2, $H_{5',5''}$), 5.70 (d, $J_{1'-2'} = 2.5$ Hz, 1, $H_{1'}$), 6.00 ("q," $J_{2'-1'} = 2.5$, $J_{2'-3'} = 4$ Hz, 1, $H_{2'}$). Careful irradiation at δ 5.70 ($H_{1'}$) collapsed the peak at δ 6.00 ($H_{2'}$) to a rough doublet. Careful irradiation at δ 6.02 ($H_{2'}$) collapsed the peak at δ 5.70 ($H_{1'}$) to a rough singlet. Irradiation at δ 4.41 ($H_{3'}$) collapsed the peak at δ 6.02 ($H_{2'}$) to a rough doublet but had no effect on the δ 5.70 ($H_{1'}$) peak.

2′-*O*-Methylguanosine[17] (5c) and 3′-*O*-Methylguanosine[18] (6c). A stirred mixture of 1.5 g (0.0053 mol) of **4c** and 0.25 g (0.0011 mol) of $SnCl_2 \cdot 2H_2O$ in 500 ml of DMF at 50–55° was treated dropwise with 26 ml of CH_2N_2 stock solution A over a 6-hr period. At this point only traces (tlc) of **4c** remained and the solution was cooled and evaporated to about 15 ml. MeOH was added until the solution became turbid, and 15 g of silica gel (A) was added. The mixture was evaporated to dryness and the impregnated powder was applied to a column (52 × 3.5 cm, 100 g) of the same silica gel. The column was eluted with 1000 ml of $CHCl_3$ followed by MeOH–$CHCl_3$ (15:85) at a rate of 1.7 ml/min and 15-ml fractions were collected. Fractions 101–170 contained only **5c** and **6c.** This combined fraction was evaporated to dryness to give 0.5 g of solid. Fractions 86–100 were combined, evaporated, absorbed on silica gel, and rechromatographed on a column (1.8 cm diameter, 20 g) of the same silica gel. $CHCl_3$ containing 1% ammonia was used to elute the more rapidly migrating impurity.

MeOH–MeOAc (1:1) was used to elute 0.075 g of chromatographically homogeneous **5c** and **6c**. Fractions 171–250 were treated as fractions 86–100 [except that MeOH–MeOAc (1:1) was the only elution solvent] to provide an additional 0.18 g of **5c** and **6c** for an overall combined yield of 0.755 g (48%).

A column (150 × 3.5 cm, 1000 ml) of Dowex 1-X2 (Cl^-) 200–400 mesh) packed in distilled H_2O was washed with 500 ml of 2 *N* NH_4OH. A solution of 0.6 g of **5c** and **6c** in 5 ml of 2 *N* NH_4OH was applied to the column followed by 100 ml of 2 *N* NH_4OH. The column was developed with 0.02 *N* NH_4Cl brought to pH 10.00 ± 0.03 by the addition of 6.62 ml of concentrated NH_4OH per 1000 ml of 0.02 *N* NH_4Cl solution. The flow rate of the buffer through the column was 1.95 ml/min and 24.5-ml fractions were collected. Fractions 1–180 contained minor spots (tlc) and were discarded. Fractions 181–1710 were uv transparent and were discarded. Fractions 1711–1960 contained **6c**. Fractions 1961–2100 were uv transparent and were discarded. Fractions 2101–2470 contained **5c**.

Pooled fractions 1711–1960 were stirred for 15 hr with 1.25 g of activated charcoal (Barnebey-Cheney AU-4 prewashed with 6 *N* HCl and distilled H_2O and dried). The mixture was filtered, the uv-transparent filtrate was discarded, and the charcoal was continuously extracted with 450 ml of EtOH–concentrated NH_4OH (7:3) for 24 hr in a Soxhlet apparatus. Two additional 24-hr extractions with fresh solvent were effected and the pooled extracts were evaporated to give 0.18 g (14% overall from **4c**) of crystalline **6c**. A sample was recrystallized from MeOH to give fine needles: mp >258° dec; $[\alpha]^{25}D$ −74.7° (*c* 0.36, 0.1 *N* NaOH); uv (H_2O) max 253 nm (ε 14,000), min 223 nm (ε 3800) [lit.[18] mp 263–300° dec; $[\alpha]^{25}D$ −69° (*c* 1, 0.1 *N* NaOH)].

Anal. Calcd for $C_{11}H_{15}N_5O_5$: C, 44.44; H, 5.09; N, 23.56. Found: C, 44.30; H, 5.19; N, 23.50.

Fractions 2101–2470 from the Dowex 1-X2 (Cl^-) column were pooled, stirred with 2 g of activated charcoal, and continuously extracted with four 150–200-ml portions of EtOH–concentrated NH_4OH (7:3) as described above for **6c**. Evaporation of the extracts gave 0.19 g (15% overall from **4c**) of crystalline **5c**. A sample was recrystallized from MeOH to give **5c**·MeOH: mp 234–236° (resolidified and darkened at >240°); $[\alpha]^{25}D$ −52.0° (*c* 0.35, 0.1 *N* NaOH); uv (H_2O) max 254 nm (ε 13,400), min 224 nm (ε 2400) [lit.[17] mp 218–220°; $[\alpha]^{22}D$ − 38.4° (*c* 0.595, H_2O)].

Anal. Calcd for $C_{11}H_{15}N_5O_5 \cdot CH_3OH$: C, 43.76; H, 5.81; N, 21.27. Found: C, 43.54; H, 5.54; N, 21.73. The presence of CH_3OH was verified by integration of the methyl signal in the nmr spectrum.

2′-*O*-Methylcytidine[12,15] (5d) and 3′-*O*-Methylcytidine[12,15] (6d). A mixture of 0.49 g (0.002 mol) of **4d** and 0.013 g of $SnCl_2 \cdot 2H_2O$ in 120 ml of MeOH was treated with 9 ml of CH_2N_2 stock solution B according to the general methylation procedure. The evaporation residue was dissolved in 5 ml of H_2O and applied to a column (58 × 2.5 cm) of Dowex 1-X2 (OH^-). The column was packed and eluted with distilled H_2O and 5-ml fractions were collected at about 1 ml/min. Fractions 141–155 contained **5d**, 156–173 were uv transparent and were discarded, and 174–220 contained **6d**.

Fractions 141–155 were pooled and evaporated to give 0.38 g (74%) of crystalline **5d**, mp 251–254°. A sample was recrystallized from EtOH to give **5d**: mp 255–257°; $[\alpha]^{22}D$ 72.3° (*c* 1.1, H_2O); uv (H_2O) max 271 nm (ε 9000), min 250 nm (ε 6300); uv (0.1 *N* HCl) max 279 nm (ε 13,200), min 240 nm (ε 1800); uv (0.1 *N* NaOH) max 271 nm (ε 9500), min 250 nm (ε 6600).

Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.70; H, 5.80; N, 16.30. Found: C, 46.99; H, 5.87; N, 16.31.

Analogous work-up of fractions 174–220 gave 0.083 g (15%) of **6d**: mp 210–211°; $[\alpha]^{22}D$ 58.2° (*c* 1, H_2O); uv (H_2O) max 270 nm (ε 8600), min 248 nm (ε 5900); uv (0.1 *N* HCl) max 279 nm (ε 13,400), min 239 nm (ε 1500); uv (0.1 *N* NaOH) max 270 nm (ε 9200), min 248 nm (ε 6500).

Anal. Found: C, 46.63; H, 5.70; N, 16.26.

(See ref 12 and 15 for comparisons.)

4-Methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone[8] (5e) and 4-Methoxy-1-(3-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (6e). Method A. A solution of 0.52 g (0.002 mol) of **4e** in 120 ml of 10^{-3} *M* $SnCl_2 \cdot 2H_2O$ in MeOH was treated with 12 ml of CH_2N_2 stock solution B. The evaporation residue was crystallized directly from dry, acid-free EtOAc to give 0.31 g (57%) of pure **5e**, mp 171– 172°, identical in every respect with a known analytically pure sample.[8]

Method B. A solution of 1 g (0.004 mol) of **4e** and 0.08 g (0.0004 mol) of $SnCl_2 \cdot 2H_2O$ in 300 ml of MeOH was treated with 50 ml of CH_2N_2 stock solution A according to the general methylation procedure. The evaporation residue was dissolved in a small volume of MeOH, and 5 g of silica gel (A) was added. The mixture was evaporated and the impregnated powder was applied to a column (1.8 cm diameter, 50 g) of silica gel (B). The column was developed with MeOAc–*n*-C_5H_{12}–MeOH (84:14:0.5) at a rate of 0.33 ml/min and 5-ml fractions were collected. Fractions 101–200 contained pure **5e** and were evaporated to give 0.55 g of crystalline **5e**, mp 170–173°. Fractions 201–400 contained **5e** and **6e** and were evaporated to give 0.48 g of gum. The 0.48 g of mixed isomers was dissolved in MeOH and applied to four silica gel preparative tlc plates (20 × 20 cm). The plates were developed in MeOAc–*n*-C_5H_{12}–MeOH (84:14:0.5) three times for 1 hr each time with 15 min of drying between each development. The more rapidly migrating band was scraped off, extracted with MeOH, and evaporated to give 0.23 g of crystalline **5e**, mp 170–174°, total yield of **5e**[8] 0.78 g (74%).

The slower band was treated analogously to give 0.16 g (15%) of crystalline **6e**, mp 127–130°. This product was recrystallized from EtOH–Skellysolve B to give **6e**: mp 142–144°; $[\alpha]^{23}D$ 107.9° (*c* 1, H_2O); uv (H_2O) max 274 nm (ε 6900), min 238 nm (ε 1200); uv (0.1 *N* HCl) max 274 nm (ε 7000), min 237 nm (ε 1800); uv (0.1 *N* NaOH) max 275 nm (ε 7000), min 241 nm (ε 1900).

Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.23; H, 5.76; N, 10.39.

2′-*O*-Methyluridine[6,8,12,15,16] (5f) and 3′-*O*-Methyluridine[6,12,15] (6f). A solution of 0.73 g (0.003 mol) of **4f** and 0.068 g (0.0003 mol) of $SnCl_2 \cdot 2H_2O$ in 300 ml of MeOH was treated dropwise with 110 ml of CH_2N_2 stock solution A over a period of 2 hr according to the general methylation procedure. The evaporation residue was dissolved in MeOH and applied to six alumina preparative tlc plates (20 × 40 cm). The plates were developed in $CHCl_3$–MeOH–Me_2CO–H_2O (120:60:23:14) for 8 hr, dried for 15 min, and redeveloped for 2 hr in the same solvent system. The more rapidly migrating major band was scraped off and continuously extracted with 200-ml portions of MeOAc–MeOH (4:1) in a Soxhlet apparatus three times for 24 hr each. The combined extracts were evaporated to give 0.45 g (58%) of **5f**, mp 155–158°. A sample was recrystallized from EtOH–EtOAc to give **5f**, mp 159–161°, identical with a known sample.[8]

Anal. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.50; H, 5.47; N, 10.38. Found: C, 46.22; H, 5.65; N, 10.52.

The slower migrating major band was treated analogously to give 0.22 g (28%) of **6f** as a tlc-homogeneous glass with all spectral properties corresponding to this structure (see Tables I and II). Several attempts to crystallize this product were unsuccessful, as also noted by Shugar.[12] The conversion to its crystalline diacetyl derivative in high yield verified the purity of this glassy sample of **6f** as follows.

3′-*O*-Methyl-2′,5′-di-*O*-acetyluridine. A solution of 2 ml of pyridine and 10 ml of Ac_2O was added to 0.16 g (0.006 mol) of glassy **6f** and the resulting solution was stirred at room temperature for 1 hr. The reaction was shown to be complete by tlc and was evaporated to dryness *in vacuo*. The residue was dissolved in $CHCl_3$ and applied to a silica gel preparative tlc plate (20 × 20 cm). The plate was developed with $CHCl_3$–EtOAc (2:1), and the band was scraped off, extracted with MeOAc, and evaporated. The resulting glass was crystallized in two crops from EtOH–Skellysolve B to give 0.20 g (94%) of anytically pure 3′-*O*-methyl-2′,5′-di-*O*-acetyluridine: mp 150–152°; $[\alpha]^{23}D$ 34.7° (*c* 0.96, MeOH); uv (MeOH) max 258 nm (ε 9500), min 227 nm (ε 2100); uv (0.1 *N* HCl in MeOH) max 258 nm (ε 9300), min 227 nm (ε 1800); uv (0.1 *N* NaOH in MeOH) max 260 nm (ε 6600), min 237 nm (ε 4700); nmr ($CDCl_3$, TMS internal) δ 2.13 and 2.17 (s and s, 3 and 3, 2′- and 5′-$OCOCH_3$), 3.39 (s, 3, 3′-OCH_3), 3.92 ("t," $J_{3'-2'}$ = 5, $J_{3'-4'}$ = 7.5 Hz, 1, $H_{3'}$), 4.18 (m, 1, $H_{4'}$), 4.35 ("d," 2, $H_{5',5''}$), 5.37 ("q," $J_{2'-1'}$ = 3.5, $J_{2'-3'}$ = 5 Hz, 1, $H_{2'}$), 5.64 (d, J_{5-6} = 8 Hz, 1, H_5), 5.81 (d, $J_{1'-2'}$ = 3.5 Hz, 1, $H_{1'}$), 7.40 (d, J_{6-5} = 8 Hz, 1, H_6), 8.71 (br s, 1, N_3-H).

Anal. Calcd for $C_{14}H_{18}N_2O_8$: C, 49.12; H, 5.30; N, 8.18. Found: C, 48.95; H, 5.44; N, 7.93.

5-(2-*O*-Methyl-β-D-ribofuranosyl)uracil (2′-*O*-Methylpseudouridine, 8) and 5-(3-*O*-Methyl-β-D-ribofuranosyl)uracil (3′-*O*-Methylpseudouridine, 9). To a stirred mixture of 0.24 g (0.001 mol) of 7[38] and 0.046 g of $SnCl_2 \cdot 2H_2O$ in 135 ml of MeOH was added a total of 49 ml of diazomethane stock solution A dropwise at such a rate that yellow color did not persist. Complete reaction was indicated (tlc) and a 1-g portion of silica gel (B) was added. The mixture was evaporated to dryness and the impregnated powder was applied to a column (1.2 cm diameter, 16 g) of the same absorbent. The column was developed with MeOAc–$CHCl_3$–EtOH (10:1:0.1) at a rate of 1.5 ml/min. Fractions of 3.2

ml were collected. Fractions 5–110 contained multiply methylated products (tlc) which were not identified. Fractions 141–180 were found (upon standing) to contain 0.012 g of slender needles of **8**, mp 214–217°, which were collected by decantation of solvent and scratching from the test tubes. The adhering crystals were dissolved in MeOH, pooled, evaporated, and crystallized from EtOH–EtOAc to give 0.034 g of needles, mp 209–212°. The mother liquor was combined with the decanted elution solvent and evaporated to dryness. Crystallization of this residue from EtOH–EtOAc gave 0.023 g of **8**, mp 200–210°, total collected yield of **8** 0.069 g (27%). The needles of **8**, mp 214–217°, had $[\alpha]^{23}D$ 22.2° (*c* 0.93, H_2O); uv (H_2O) max 263 nm (ϵ 7800), min 232 nm (ϵ 1500); uv (0.1 *N* HCl) max 263 nm (ϵ 8100), min 232 nm (ϵ 2000); uv (0.1 *N* NaOH) max 287 nm (ϵ 8100), min 246 nm (ϵ 2500).

Anal. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.50; H, 5.47; N, 10.38. Found: C, 46.62; H, 5.70; N, 10.30.

A 9-mg sample of **8** was trifluoroacetylated using 0.5 ml of trifluoroacetic anhydride and 2 ml of dry CH_2Cl_2 at 5° for 20 hr by the general procedure to give a residue which had nmr (Me_2CO—d_6, TMS internal) δ 3.40 (s, 3, 2-OCH_3), 4.50 (m, 2, $H_{2'}$ and $H_{4'}$), 4.73 (m, 3, $H_{1'}$ and $H_{5',5''}$), 5.59 ("t," $J_{3'-2'} \cong J_{3'-4'}$ = 5.5 Hz, 1, $H_{3'}$), 6.48 (br s, 2, N_1 H and N_3 H), 7.67 (s, 1, H_6). Irradiation at δ 5.58 ($H_{3'}$) simplified the high-field multiplet ($H_{2'}$ and $H_{4'}$ but did not affect the multiplet centered at δ 4.73 ($H_{1'}$ and $H_{5',5''}$). Irradiation at δ 4.72 ($H_{1'}$ and $H_{5',5''}$) did not affect the "triplet" at δ 5.59 ($H_{3'}$). Irradiation at δ 4.54 ($H_{2'}$ and $H_{4'}$) collapsed the triplet at δ 5.59 to a singlet and simplified the multiplet at δ 4.73 to two peaks.

Fractions 181–340 from the above column contained both **8** and **9** and were pooled and evaporated to a small volume. A 1-g portion of silica gel (B) was added and the mixture was evaporated to dryness. The impregnated powder was applied to a similar column, which was developed with MeOAc–$CHCl_3$–EtOH (10:1:0.2). Fractions (3.2 ml) 31–110 contained 0.11 g (43%) of mixed **8** and **9** (as a dried solid after evaporation of solvents). Fractions 111–220 were pooled and evaporated to give 0.026 g (10%) of crystalline **9**: mp 225–228° dec; uv (H_2O) max 262 nm (ϵ 6500), min 232 nm (ϵ 1900); uv (0.1 *N* HCl) max 262 nm (ϵ 6900), min 233 nm (ϵ 2500); uv (0.1 *N* NaOH) max 287 nm (ϵ 6500), min 246 nm (ϵ 2300); mass spectrum *m/e* 259.0930 [calcd for $C_{10}H_{15}N_2O_6$ (M + 1), 259.0916].

Anal. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.50; H, 5.47; N, 10.38. Found: C, 46.63; H, 5.64; N, 10.27.

A 10-mg sample of **9** was trifluoroacetylated by the general procedure. The evaporation residue gave nmr (Me_2CO-d_6, TMS internal) δ 3.37 (s, 3, 3′-OCH_3), 4.15 (m, 1, $H_{4'}$), 4.17 (d, $J_{3'-2'}$ = 4 Hz, $J_{3'-4'}$ small, 1, $H_{3'}$), 4.68 (m, 2, $H_{5',5''}$), 4.93 (d, $J_{1'-2'}$ = 3 Hz, $H_{1'}$), 5.74 ("q," $J_{2'-1'}$ = 3, $J_{2'-3'}$ = 4 Hz, 1, $H_{2'}$), 7.57 (br s, 1, H_6). Irradiation at δ 5.78 ($H_{2'}$) caused collapse of the doublet at δ 4.93 ($H_{1'}$) to a singlet and that at δ 4.17 ($H_{3'}$) to a singlet. Irradiation at δ 4.95 ($H_{1'}$) caused collapse of the band at δ 5.74 ($H_{2'}$) to a doublet ($J_{2'-3'}$ = 4 Hz). Irradiation at δ 4.19 ($H_{3'}$) caused collapse of the band at δ 5.74 ($H_{2'}$) to a doublet ($J_{2'-1'}$ = 3 Hz) but had no effect on the δ 4.93 ($H_{1'}$) doublet. Irradiation at δ 4.71 ($H_{5',5''}$) raised a singlet at δ 4.12 ($H_{4'}$), confirming the coupling of these bands.

Registry No.—**1a**, 58-61-7; **1b**, 5399-87-1; **2a**, 2140-79-6; **2b**, 51293-29-9; **2c**, 13039-46-8; **2d**, 51293-30-2; **3a**, 10300-22-8; **3b**, 51293-31-3; **3c**, 10300-25-1; **3d**, 51293-32-4; **4a**, 69-33-0; **4b**, 6742-12-7; **4c**, 118-00-3; **4d**, 65-46-3; **4e**, 34218-77-4; **4f**, 58-96-8; **5a** monohydrochloride, 51293-33-5; **5a** trifluoroacetate, 51293-34-6; **5b** monohydrochloride, 51293-35-7; **5b** trifluoroacetate, 51364-38-6; **5c**, 2140-71-8; **5d**, 2140-72-9; **5e**, 34218-78-5; **5f**, 2140-76-3; **6a**, 51293-36-8; **6a** trifluoroacetate, 51293-37-9; **6b** monohydrochloride, 51364-39-7; **6b** trifluoroacetate, 51293-38-0; **6c**, 10300-27-3; **6d**, 20594-00-7; **6e**, 34202-79-4; **6f**, 6038-59-1; **7**, 1445-07-4; **8**, 2140-68-3; **8** trifluoroacetate, 51293-39-1; **9**, 51293-40-4; **9** trifluoroacetate, 51293-41-5; 3′-*O*-methyl-2′,5′-di-*O*-acetyluridine, 51293-42-6.

References and Notes

(1) (a) This work was generously supported by the National Cancer Institute of Canada, the National Research Council of Canada (A5890), and the University of Alberta. (b) For the previous paper in this series, see M. J. Robins, Y. Fouron, and R. Mengel, *J. Org. Chem.*, **39**, 1564 (1974).
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