Nucleic Acid Related Compounds. 1. Methylation and Transformation of 4-Methoxy-2-pyrimidinone 1-β-D-Ribofuranoside into 2′-O-Methyl Nucleoside Components of Ribonucleic Acid, Their Analogs, and Derivatives*

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ABSTRACT: Methylation of 4-methoxy-1-β-D-ribofuranosyl-2-pyrimidinone (**1**) with diazomethane gave 4-methoxy-1-(2-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**), 4-methoxy-1-(3-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**3**), and 4-methoxy-1-(2,3-di-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**4**). The pure 2′-O-methyl product (**2**) was isolated in 37% yield under selected conditions. Acid hydrolysis of **2** gave 2′-O-methyluridine (**5**) whereas nucleophilic displacement of the 4-methoxyl function by ammonia or methylamine gave 2′-O-methylcytidine (**7**) and the new RNA minor component N^4-methyl-2′-O-methylcytidine (**8**), respectively. Replacement with hydrosulfide gave 2′-O-methyl-4-thiouridine (**6**) and reaction of **2** or **5** under the appropriate halogenating conditions gave 5-chloro- (**11**), 5-bromo- (**12**), and 5-iodo-2′-O-methyluridine (**13**). The 2′,3′-di-O-methyl nucleoside (**4**) could be prepared almost exclusively by extended treatment of **1** with excess diazomethane. The syntheses of 2′,3′-di-O-methyluridine (**14**) and cytidine derivatives from this intermediate are described.

We wish to report a general procedure for the synthesis of 2′-O-methyl nucleosides of 4-substituted 2-pyrimidinones. The 3′-O-methyl and 2′,3′-di-O-methyl derivatives can also be obtained from the reaction mixture.

Numerous reports (see, for example, Smith and Dunn, 1959, Hall, 1964, Tamaoki and Lane, 1968, Lane and Tamaoki 1969, and references therein) have appeared concerning the widespread occurrence of 2′-O-methyl nucleosides in RNA from various sources. It is therefore of considerable interest to have quantities of authentic synthetic samples for comparison and study.

Although it has been shown (Nichols and Lane, 1968; Svensson *et al.*, 1968) that $O^{2'}$-methylation of nucleosides can occur at the polynucleotide level after polymer synthesis, it has also been observed (Janion *et al.*, 1970) that pyrimidine 2′-O-methyl nucleosides can be enzymatically phosphorylated to the mononucleotide level. The corresponding diphosphates can also be enzymatically polymerized to the polynucleotide level (Rottman and Johnson, 1969; Janion *et al.*, 1970; Zmudzka and Shugar, 1970). Several recent studies on polynucleotides containing large quantities of 2′-O-methyl groups (see, for example, Bobst *et al.*, 1969a,b, Rottman and Johnson, 1969, Janion *et al.*, 1970, Żmudzka and Shugar, 1970, and references therein) have demonstrated that these polymers as well as monomers (Janion *et al.*, 1970) behave like ribo- rather than deoxyribonucleotides. Studies on the enhancement of lysyl-tRNA binding to ribosomes by tri- and tetranucleotide units have shown that the 2′-O-methyloligoadenylates function as ribonucleotide templates whereas the 2′-deoxyoligoadenylates are essentially inactive (Price and Rottman, 1970). Thus the 2′-O-methyl group is a unit of considerable interest and versatility in the chemistry and binding of nucleic acid structures. It has been reported that the 2′-O-methyl nucleoside derivatives differ significantly from the corresponding nucleoside derivatives in being resistant to hydrolase and phosphorylase enzymes (Honjo *et al.*, 1964; Hudson *et al.*, 1965). Glycosidic bond cleavage is an important mechanism (Heidelberger, 1965) in the cross-resistance of a biochemically active base and its nucleoside and thus the synthesis of 2′-O-methyl nucleosides of active bases is also a significant area for investigation. The report by Honjo and coworkers (1964) that the 2′-O-methyl nucleoside 5′-phosphates are not substrates for bull semen 5′-nucleotidase would also appear to protect the emergence of any biochemically active 2′-O-methyl nucleotide from inside the cell. However, a snake venom 5′-nucleotidase has been reported by Shugar's laboratory (Janion *et al.*, 1970) to dephosphorylate 2′-O-methylcytidine 5′-phosphate and thus a reexamination of this enzyme activity or specificity appears to be in order (see also Hudson *et al.*, 1965).

The somewhat laborious synthesis of 2′-O-methyluridine and -cytidine and 3′-O-methyluridine and -cytidine (by methyl iodide–silver oxide methylation of 3′,5′-di-O-trityluridine and 2′,5′-di-O-trityluridine, respectively, followed by separation of N^3- from O^4-ring-methylated products and subsequent chemical transformations) has been reported (Furukawa *et al.*, 1965). A direct methylation of cytidine (Martin *et al.*, 1968) followed by an extensive purification scheme gave 2′-O-methylcytidine in 14% yield plus additional small quantities of purified 2′-O- and 3′-O-methylcytidines. These products were deaminated to the corresponding 2′-O- and 3′-O-methyluridines.

We have sought a pyrimidine 2′-O-methyl nucleoside intermediate which could be readily transformed into the natural product ribonucleic acid components as well as biochemically interesting analogs.

Treatment of 4-chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-pyrimidinone (Žemlička and Šorm, 1965) with

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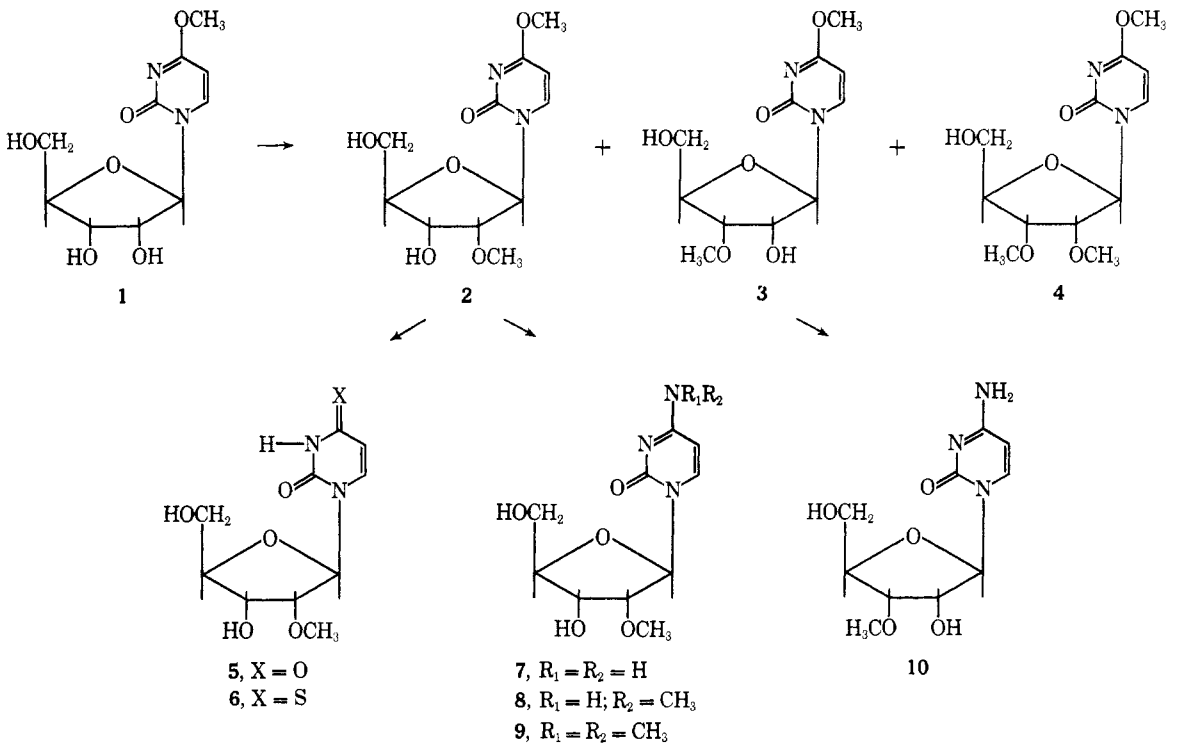
SCHEME I



excess sodium methoxide in methanol gave pure 4-methoxy-1-β-D-ribofuranosyl-2-pyrimidinone (**1**) (see Scheme I) in 74% yield from the crude chloro product. It is of interest that this is apparently the first isolation and characterization of the useful intermediate, **1**, although its precursors and analogs are versatile intermediates in the Hilbert–Johnson synthesis of pyrimidine nucleosides (see, for example, Hilbert and Johnson, 1930, Hilbert, 1937, Keller and Tyrrill, 1966, Prystaš, 1967, and references therein).

This product (**1**) was methylated with excess diazomethane in 1,2-dimethoxyethane–water (Broom and Robins, 1965) at *room temperature* to give a 37% yield of 4-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**) after column chromatography and crystallization. An 18% yield of pure 4-methoxy-1-(2,3-di-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**4**) was also obtained by crystallization of the appropriate column fractions. The pmr spectrum of the crude sugar-monomethylation fractions showed a 2′- to 3′-O-methylation ratio of about 3.5:1. This was verified by amination of the mother liquor and chromatographic separation and identification of the known (Furukawa *et al.*, 1965; Martin *et al.*, 1968) 2′-*O*-methylcytidine (**7**) and 3′-*O*-methylcytidine (**10**).

Treatment of 4-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**) with Dowex 50W-X8 (H^+) resin in water gave a smooth conversion (95% yield) into 2′-*O*-methyluridine (**5**) (Furukawa *et al.*, 1965; Martin *et al.*, 1968). Reaction of **2** with alcoholic ammonia in a sealed tube gave an 89% yield of 2′-*O*-methylcytidine (**7**) (Furukawa *et al.*, 1965; Martin *et al.*, 1968). These transformations provide an alternative synthesis of the two natural products **5** and **7** and firmly establish the structures of **2**, **5**, **7**, and **10** (as well as the remaining analogs).

Nichols and Lane (1966) reported the isolation of N^4-methyl-2′-*O*-methylcytidine (**8**) from ribosomal and soluble RNA which represented an example of a new class of minor component with both base and sugar methylated. This new natural component (**8**) was prepared in 83% yield by stirring **2** with an alcoholic methylamine solution. Analogous reaction of **2** with alcoholic dimethylamine gave N^4,N^4-dimethyl-2′-*O*-methylcytidine [4-*N*,*N*-dimethylamino-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone] (**9**).

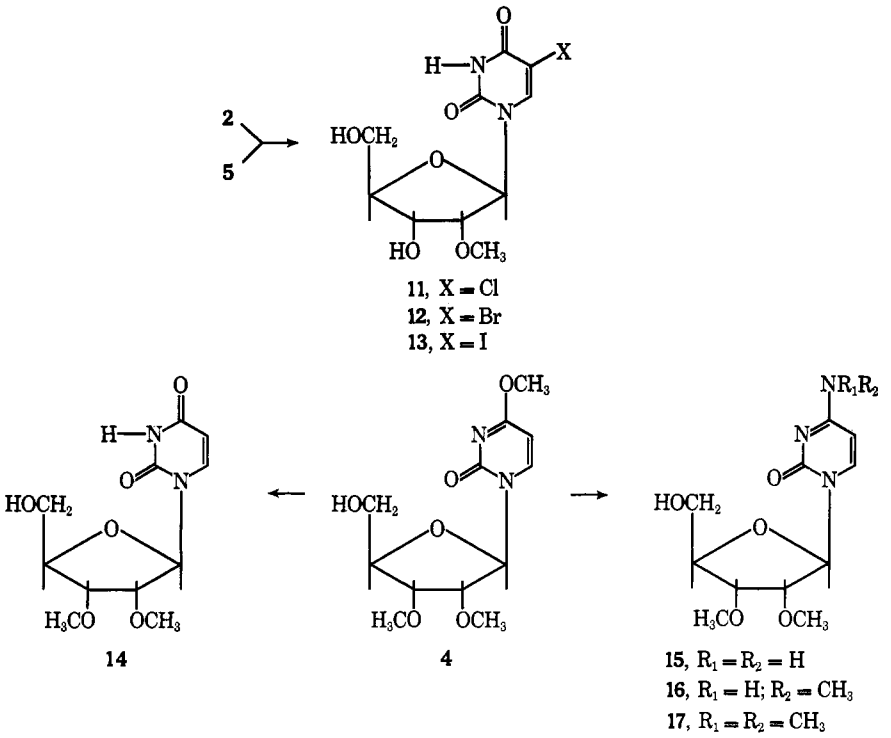
Reaction of **2** with alcoholic sodium hydrosulfide produced 2′-*O*-methyl-4-thiouridine (**6**). This compound is seen to be the 2′-*O*-methyl derivative of the interesting minor component nucleoside 4-thiouridine (Lipset, 1965; Ziff and Fresco, 1968; Hayatsu and Yano, 1969). It is of interest to note that 4-thiouridine, a tRNA natural product, has been found to exhibit biochemical inhibition of certain cell lines (Bloch *et al.*, 1969).

The 5-halouracil nucleosides and 2′-deoxynucleosides have been shown to have pronounced biochemical activity in tumor (Heidelberger, 1965) and viral systems (Prusoff, 1967).

In view of the altered enzymatic properties reported for 2′-*O*-methyl nucleoside derivatives (Honjo *et al.*, 1964; Hudson *et al.*, 1965), the investigation of 5-halo-2′-*O*-methyluridines is of significant interest. Treatment of 2′-*O*-methyluridine (**5**) (Scheme II) or alternatively 4-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**) with chlorine in acetic acid–carbon tetrachloride (Prystaš and Šorm, 1964) gave 5-chloro-2′-*O*-methyluridine (**11**) in excellent yield. The action of bromine water (Prystaš and Šorm, 1964) on **2** or **5** gave high yields of 5-bromo-2′-*O*-methyluridine (**12**) and iodination (Prusoff *et al.*, 1953) produced 5-iodo-2′-*O*-methyluridine (**13**) in approximately 70% yield.

It has been shown (Toji and Cohen, 1969, 1970; Atkinson *et al.*, 1969) that 2′,3′-dideoxyadenosine (Robins and Robins,

SCHEME II



1964) triphosphate and other 2′,3′-dideoxynucleotides can function as DNA chain-terminating agents and can be enzymatically polymerized onto the end of a growing DNA chain. Therefore, we have prepared certain 2′,3′-di-*O*-methyluridine and -cytidine derivatives for evaluation of their biochemical and physical properties.

Treatment of **1** with a larger excess of diazomethane over a prolonged reaction period gave 4-methoxy-1-(2,3-di-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**4**) as the major reaction product with a small amount of sugar monomethylated products and a trace of starting material (**1**). The 4-, 2′-, and 3′-*O*-methyl resonances in the pmr spectra of **4** were comparable to the corresponding peaks in the 4-methoxy-1-(2′- and 3′-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinones (**2** and **3**) and a cleanly resolved triplet was observed for the 5′-CH_2OH hydroxyl proton in a spectrum determined in Me_2SO. In addition, further methylation of a known mixture of **2** and **3** gave **4** and thus the structure is clearly that of a 2′,3′-di-*O*-methyl derivative.

Reaction of **4** with Dowex 50W-X8 (H^+) resin in aqueous methanol gave 2′,3′-di-*O*-methyluridine (**14**) in 75% yield. The physical properties of **14** are similar to that of a compound reported by Levene and Tipson (1934) from methylation of 5′-*O*-trityluridine followed by acid hydrolysis. In our hands, the Levene–Tipson procedure gave a mixture of N^3- and O^4-methylated products analogously to the results of Furukawa *et al.* (1965). Treatment of **4** with ammonia, methylamine, and dimethylamine produced 2′,3′-di-*O*-methylcytidine (**15**), N^4-methyl-2′,3′-di-*O*-methylcytidine [4-*N*-methylamino-1-(2,3,di-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone] (**16**), and N^4,N^4-dimethyl-2′,3′-di-*O*-methylcytidine [4-*N*,*N*-dimethylamino-1-(2,3-di-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone] (**17**), respectively.

Further studies on the synthesis of other 2′-*O*-methyl-nucleosides of current biochemical interest and a facile methylation procedure will be reported separately.

Experimental Section

Melting points were determined on a Kofler micro melting point apparatus and are uncorrected. Nmr spectra were determined on a Varian A-60 instrument with tetramethylsilane or sodium 5,5-dimethyl-5-silapentanesulfonate as internal reference. Uv spectra were determined by dissolving an accurately weighed sample in water or methanol and then diluting a 1-ml portion of this stock solution to 10 ml with 0.1 N HCl, freshly prepared 0.1 N NaOH solution, or distilled water and running the spectrum on a Cary 14M recording spectrophotometer as rapidly as possible. The acidic spectra of the 4-methoxy-2-pyrimidinone nucleosides and the basic spectra of the 5-halouridines are time labile and should be accepted with reservation. Evaporations were accomplished using a Büchler rotating evaporator under reduced pressure (aspirator) and at 40° or lower unless specified otherwise. Optical rotations were determined on a Perkin-Elmer Model 141 digital readout polarimeter using a 1-cc, 10-cm micro cell. Thin-layer chromatography was run on Eastman Chromatogram sheet (silica gel with indicator 6060 or Alumina with indicator 6063) using the solvent specified. Solvents and chemicals were of reagent quality unless specified.

4-Chloro-1-(2,3,5-tri-O-benzoyl-β-D-ribofuranosyl)-2-pyrimidinone. This product was prepared according to Žemlička and Šorm (1965) from 33.3 g (0.06 mole) of 2′,3′,5′-tri-*O*-benzoyluridine (Prystaš and Šorm, 1964), 48 ml (0.67 mole) of thionyl chloride (Eastman White Label), and 3 ml of reagent dimethylformamide in 300 ml of chloroform. The solution was refluxed with stirring for 6 hr and then evaporated and the residual solid dried *in vacuo* (oil pump) for 15 hr at room

temperature. The pale yellow solid was triturated with 50 ml of dry benzene and collected by filtration. This product (30.9 g, 90%) had mp 195–205°, lit. (Žemlička and Šorm, 1965) mp 204–205°, and was suitable for the next step without further purification.

4-Methoxy-1-β-D-ribofuranosyl-2-pyrimidinone (**1**). To a solution of 30.9 g (0.0537 mole) of the above crude chloro compound in 500 ml of dry methanol was added 2.75 g (0.119 g-atom) of sodium dissolved in 200 ml of dry methanol. The resulting solution was refluxed for 20 min and allowed to stand for 15 hr at room temperature protected from moisture by a drying tube at all times. Amberlite IRC-50 resin (50 ml) was added and stirred until the mixture was neutral (damp pH paper). The resin was filtered and washed well with methanol. The combined filtrate was evaporated to a yellow oil which was diluted with 150 ml of water and washed with four 25-ml portions of ether and one 25-ml portion of chloroform. The aqueous layer was evaporated to dryness (oil pump) and the residue was coevaporated twice with absolute ethanol. The resulting residue was extracted with six 400-ml portions of boiling (dry, acid free) ethyl acetate. The combined ethyl acetate extract was evaporated to give a chromatographically pure colorless solid, mp 134–136° (11.7 g, 84.5%), suitable for the next step. Recrystallization of a 1-g sample of the above solid from ethyl acetate gave 0.88 g of needles: mp 141–142°; $[\alpha]_D^{25}$ 78.3° (*c* 2, H_2O); uv (0.1 N HCl) max 271 nm (ε 6890), min 235 nm (ε 2100); (H_2O) max 274 nm (ε 7060), min 239 nm (ε 1500); (0.1 N NaOH) max 274 nm (ε 6710), min 239 nm (ε 1700); nmr (D_2O) τ 1.80 (d, 1, J_{6-5} = 7.5 Hz, H_6), 3.75 (d, 1, J_{5-6} = 7.5 Hz, H_5), 4.06 (d, 1, $J_{1'-2'}$ = 2.5 Hz, $H_{1'}$), 5.68–6.09 (complex, 5, $H_{2',3',4',5',5''}$),[1] 6.03 (s, 3, 4-OCH_3).

Anal. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.28; H, 5.18; N, 10.89.

Reaction of Diazomethane on 4-Methoxy-1-β-D-ribofuranosyl-2-pyrimidinone (**1**). Diazomethane in 1,2-dimethoxyethane was prepared essentially by the procedure of Khwaja and Robins (1966). To a stirred solution of 310 ml of 40% aqueous potassium hydroxide and 650 ml of 1,2-dimethoxyethane was added 125 g of slightly damp *N*-nitrosomethylurea portionwise over about 10 min at 0–10°. After stirring for an additional 15 min, the supernatant yellow solution was separated from the aqueous layer and dried for 15 hr over potassium hydroxide pellets.

The above diazomethane solution was added in portions to a stirred solution of 12.9 g (0.05 mole) of **1** in 100 ml of water at room temperature. Progress of methylation was followed by tlc (silica gel, 6% methanol in chloroform). Initially starting material **1** and monomethylated sugar products were observed. After further addition of diazomethane, a faster spot corresponding to 4-methoxy-1-(2,3-di-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**4**) appeared. Continued addition of diazomethane and stirring for 2 days gave **4** almost exclusively.

In a reaction in which a total of 650 ml of the stock diazomethane solution was added over a 24-hr period, the following results were obtained: the excess diazomethane was removed by bubbling dry air through the solution and the resulting colorless solution was evaporated to dryness. The residue was dissolved in 100 ml of methanol, 200 g of alumina was added, and the mixture was evaporated to dryness. This material was applied to the top of a dry packed alumina column (5 × 75 cm, 800 g), the column was eluted with 0.8% methanol in chloroform, and 10 ml fractions were collected. Fractions 111–310 contained the fast-migrating product and the following 20 fractions were blank. The solvent was then changed to 6% methanol in chloroform and 15-ml fractions were collected. Continuing fractions 371–500 contained the second fastest migrating product. Elution with 20% aqueous methanol could be used to remove a small amount of starting material **1**.

4-Methoxy-1-(2,3-di-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**4**). Fractions 111–310 from the above column were pooled and evaporated to dryness to yield a white solid which showed a large major spot and two very faint faster migrating minor spots on tlc (silica gel, 6% methanol in chloroform). This residue (3.5 g) was crystallized from 25 ml of dry, acid-free ethyl acetate to give 2.17 g plus 0.41 g of second crop for a yield of 2.58 g (18.2%) of chromatographically homogeneous crystalline **4**. A small sample was recrystallized from ethyl acetate to give needles of **4**: mp 137–138°; $[\alpha]_D^{25}$ 132.8° (*c* 2, H_2O); uv (0.1 N HCl) max 272 nm (ε 6870), min 237 nm (ε 1700); (H_2O) max 274 nm (ε 7100), min 239 nm (ε 1300); (0.1 N NaOH) max 273 nm (ε 6870), min 239 nm (ε 1700); nmr (D_2O) τ 1.80 (d, 1, *J* = 7.5 Hz, H_6), 3.76 (d, 1, *J* = 7.5 Hz, H_5), 3.98 (d, 1, *J* = 2.5 Hz, $H_{1'}$), 6.03 (s, 3, 4-OCH_3), 6.41 (s, 3, 2′-OCH_3), and 6.55 (s, 3, 3′-OCH_3); nmr (Me_2SO-d_6) τ 1.64 (d, 1, J_{6-5} = 7.5 Hz, H_6), 3.95 (d, 1, J_{5-6} = 7.5 Hz, H_5), 4.14 (d, 1, $J_{1'-2'}$ = 2.5 Hz, $H_{1'}$), 4.78 (t, 1, $J_{5'-OH-H_5',H_5''}$ = 5.0 Hz, 5′-OH), 6.15 (s, 3, 4-OCH_3), 6.53 (s, 3, 2′-OCH_3), 6.68 (s, 3, 3′-OCH_3).

Anal. Calcd for $C_{12}H_{18}N_2O_6$: C, 50.34; H, 6.34; N, 9.79. Found: C, 50.69; H, 6.40; N, 9.98.

4-Methoxy-1-(2-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**). Fractions 371–500 from the above column were evaporated to dryness to give 8.15 g of white solid which was homogeneous on tlc. Its nmr spectra indicated it to be a mixture of 2′-*O*- and 3′-*O*-methyl ethers (**2** and **3**), respectively, in a ratio of about 3.5 to 1 by integration of the appropriate signals. This total product was dissolved in 650 ml of boiling, dry, acid-free ethyl acetate and the solution was then filtered and cooled to room temperature to give 3.95 g of pure **2**. A second crop (1.01 g) obtained from concentration of the mother liquor to 300 ml was also shown by nmr spectroscopy to be the pure 2′-*O*-methyl nucleoside (**2**) raising the yield to 4.96 g (36.7%), mp 172–173°. Further recrystallization of this material failed to alter the melting point or nmr spectrum; $[\alpha]_D^{25}$ 115.7° (*c* 2, H_2O); uv (0.1 N HCl) max 271 nm (ε 6170), min 238 nm (ε 1700); (H_2O) max 272 nm (ε 6300), min 239 nm (ε 1300); (0.1 N NaOH) max 272 nm (ε 6300), min 239 nm (ε 1500); nmr (D_2O) τ 1.75 (d, 1, J_{6-5} = 7.5 Hz, H_6), 3.73 (d, 1, *J* = 7.5 Hz, H_5), 3.98 (d, 1, $J_{1'-2'}$ = 2.5 Hz, $H_{1'}$), 6.04 (s, 3, 4-OCH_3), 6.42 (s, 3, 2′-OCH_3).

Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.53; H, 5.92; N, 10.29. Found: C, 48.63; H, 5.68; N, 10.16.

Reaction of the Above Mother Liquor Containing 4-Methoxy-1-(2-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**) *and 4-Methoxy-1-(3-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone* (**3**) *with Ammonia.* A portion of the gummy residue (0.4 g) obtained by evaporation of the mother liquor from **2** was heated with 27 ml of 11% ammonia in methanol at 100° for 48 hr. The solution was evaporated to dryness and the residue was dissolved in 3 ml of water. This solution was applied to a column (2.8 × 70 cm) of Dowex 1-X2 (200–400 mesh OH^-) (Dekker, 1965) and the column was developed with water. Fractions of 4.5 ml were collected at a rate of one fraction each 6 min. Fractions 301–350 were pooled and evaporated to give

[1] At 60 MHz the resonance peaks due to the 2′, 3′, 4′, 5′, and 5″ protons occurred within the range τ 5.5–6.6 as complex multiplets. These data are omitted from the remainder of the Experimental Section.

0.12 g of 2′-*O*-methylcytidine (**7**), mp 248–252°. This product was recrystallized from ethanol to give needles, mp 255–258°, lit. (Martin *et al.*, 1968) mp 252–253°, which were identical with authentic samples prepared from **2** as well as the direct methylation of cytidine.

Fractions 361–470 gave 0.16 g of 3′-*O*-methylcytidine (**10**), mp 205–211°, which after recrystallization from ethanol gave needle clusters, mp 211–212°, lit. (Martin *et al.*, 1968) mp 211–212°, which was again identical in all respects with a sample prepared from methylation of cytidine.

If the pure 4-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**) (4.96 g, 61% of monomethylated product) is subtracted from the total crude yield of monomethylated nucleosides (8.15 g, 100%), the remaining crude product from the mother liquor (39%) multiplied by the ratio of 2′-*O*-methylcytidine (**7**) to 3′-*O*-methylcytidine (**10**) 3/7:4/7 gives an overall percentage of 77.7% 2′- to 22.3% 3′- or 3.4:1 methylation ratio which confirms the nmr analysis.

2′-O-Methyluridine (**5**). A solution of 1.09 g (0.004 mole) of **2** in 25 ml of water was stirred with 7 ml of Dowex 50W-X8 (H^+) for 1.5 hr at room temperature. The mixture was filtered and the resin was thoroughly washed with water. The combined filtrate was evaporated to dryness and twice coevaporated with absolute ethanol. The resulting solid was crystallized from 150 ml of ethyl acetate to give 0.61 g of **5**, mp 160–161°, lit. (Martin *et al.*, 1968) mp 157–158.5°, lit. (Furukawa *et al.*, 1965) mp 159°. Concentration of the mother liquor gave 0.37 g of **5**, mp 158–159°, total yield 0.98 g (95%); $[\alpha]_D^{25}$ 40° (*c* 1.6, water) [lit. (Furukawa *et al.*, 1965) $[\alpha]_D^{20}$ 41° (*c* 1.6, H_2O)]; uv (0.1 N HCl) max 261 nm (ε 8600), min 230 nm (ε 1900); (H_2O) max 261 nm (ε 9600), min 230 nm (ε 1900); (0.1 N NaOH) max 261 nm (ε 7400), min 242 nm (ε 6020); nmr (D_2O) τ 2.08 (d, 1, J_{6-5} = 8.3 Hz, H_6), 4.10 (d, 1, J_{5-6} = 8.3 Hz, H_5), 4.02 (d, 1, $J_{1'-2'}$ = 3.5 Hz, $H_{1'}$), 6.47 (s, 3, 2′-OCH_3).

Anal. Calcd for $C_{10}H_{14}N_2O_6$: C, 46.50; H, 5.47; N, 10.38. Found: C, 46.54; H, 5.32; N, 10.71.

4-Thio-2′-O-methyluridine (**6**). A solution of 1.36 g (0.059 g-atom) of sodium in 20 ml of dry methanol was saturated with hydrogen sulfide gas and 1.09 g (0.004 mole) of 4-methoxy-1-(2-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**2**) was added. The solution was refluxed with stirring for 18 hr while protected from moisture and while a slow stream of hydrogen sulfide was passed through the solution. After reaction was judged to be complete by tlc (alumina, 6% methanol in chloroform), the mixture was cooled to room temperature and deionized with Dowex 50W-X8 (H^+). The mixture was filtered and the resin was thoroughly washed with methanol. The combined filtrate was evaporated to dryness and the residue was triturated with carbon disulfide and filtered. The resulting yellow hygroscopic solid was dissolved in 15 ml of 6% methanol in chloroform and applied to a silicic acid column (3.5 × 40 cm) and eluted with the same solvent. Fractions (5 ml) were collected and fractions 1–40 were discarded. Fractions 41–155 were combined and evaporated to give 0.77 g (70%) of chromatographically homogeneous solid. This material was crystallized from benzene–ethanol (7:3) with 0.5 mole of solvation of ethanol, mp 72–74°.

Anal. Calcd for $C_{10}H_{14}N_2O_5S \cdot 0.5C_2H_5OH$: C, 44.41; H, 5.72; N, 9.42. Found: C, 44.57; H, 5.83; N, 9.16.

This product (**6**) crystallizes from water with 0.25-mole hydration: mp 96–98°, $[\alpha]_D^{25}$ 94.8° (*c* 2, CH_3OH).

Anal. Calcd for $C_{10}H_{14}N_2O_5S \cdot 0.25H_2O$: C, 43.07; H, 5.24; N, 10.05; S, 11.50. Found: C, 42.93; H, 5.37; N, 10.13; S, 11.87.

The presence of solvation was indicated in the nmr spectra of these solvates: uv (0.1 N HCl) max 332 and 244 nm (ε 20,800 and 4400), min 277 and 227 nm (ε 1500 and 3300); (H_2O) max 331 and 244 nm (ε 20,500 and 3300), min 275 and 225 nm (ε 1100 and 2000); (0.1 N NaOH) max 316 nm (ε 20,800), min 259 nm (ε 3000); nmr (of hemihydrate) (D_2O) τ 2.21 (d, 1, J_{6-5} = 7.5 Hz, H_6), 3.44 (d, 1, J_{5-6} = 7.5 Hz, H_5), 4.07 (d, 1, $J_{1'-2'}$ = 3.5 Hz, $H_{1'}$), 6.46 (s, 3, 2′-OCH_3).

Further elution of the above column with 25% methanol in chloroform gave fractions which were pooled and evaporated. Crystallization of this material from ethyl acetate gave 0.2 g (19.4%) of 2′-*O*-methyluridine (**5**), mp 159–160°, spectrally and chromatographically identical with an authentic sample.

2′-O-Methylcytidine (**7**) (Furukawa *et al.*, 1965; Martin *et al.*, 1968). A solution of 0.272 g (0.001 mole) of **2** in 20 ml of 7.4% ammonia in methanol was heated in a sealed tube for 18 hr. Solvent was removed to give a solid residue, mp 250–255°, which was crystallized from ethanol to give 0.23 g (89%) of needles, mp 258–259°; $[\alpha]_D^{25}$ 69.6° (*c* 1, H_2O); uv (0.1 N HCl) max 279 nm (ε 13,000), min 240 nm (ε 1500); (H_2O) max 271 nm (ε 8740), min 250 nm (ε 6170); (0.1 N NaOH) max 271 nm (ε 9300), min 250 nm (ε 6500); nmr (D_2O) τ 2.13 (d, 1, J_{6-5} = 7.5 Hz, H_6), 3.93 (d, 1, J_{5-6} = 7.5 Hz, H_5), 4.02 (d, 1, $J_{1'-2'}$ = 3.5 Hz, $H_{1'}$), 6.44 (s, 3, 2′-OCH_3).

Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.30. Found: C, 46.63; H, 5.82; N, 16.17.

N^4-Methyl-2′-O-methylcytidine [*4-N-Methylamino-1-(2-O-methyl-1-β-D-ribofuranosyl)-2-pyrimidinone*] (**8**). To 0.14 g (0.0005 mole) of **2** was added a cold 16% solution of methylamine in methanol and the resulting solution was allowed to stand for 5 hr at room temperature. The residue obtained after evaporating this solution to dryness was triturated with 1.5 ml of absolute methanol and filtered to give 0.11 g (83%) of **8**, mp 217–220°. A sample was recrystallized from methanol to give needles: mp 221–224°; $[\alpha]_D^{25}$ 59.2° (*c* 1, H_2O); uv (0.1 N HCl) max 281 nm (ε 13,900), min 242 nm (ε 2500); (H_2O) max 271 nm (ε 11,000), min 248 nm (ε 7800); (0.1 N NaOH) max 271 nm (ε 11,400), min 250 nm (ε 7900); nmr (D_2O) τ 2.24 (d, 1, J_{6-5} = 7.5 Hz, H_6), 3.99 (d, 1, J_{5-6} = 7.5 Hz, H_5) overlapped with 3.99 (d, 1, $J_{1'-2'} \cong$ 4 Hz, $H_{1'}$), 6.46 (s, 3, 2′-OCH_3), 7.10 (s, 3, 4-NCH_3).

Anal. Calcd for $C_{11}H_{17}N_3O_5$: C, 48.70; H, 6.32; N, 15.49. Found: C, 48.77; H, 6.10; N, 15.71.

N^4,N^4-Dimethyl-2′-O-methylcytidine [*4-N,N-Dimethylamino-1-(2-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone*] (**9**). A solution of 0.14 g (0.0005 mole) of **2** in 6 ml of methanol was treated with 3 ml of a cold 46% solution of dimethylamine in methanol. The reaction was allowed to stand at room temperature for 2 days and was then evaporated to dryness. The white residue was recrystallized from ethanol to give 0.13 g (90%) of somewhat hygroscopic crystals, mp 138–140°. This product was recrystallized from ethyl acetate to give hard crystals: mp 209–211°; $[\alpha]_D^{25}$ 49.8° (*c* 1, H_2O); uv (0.1 N HCl) max 287 nm (ε 14,500), min 246 nm (ε 2500); (H_2O) 278 nm (ε 12,400), min 238 nm (ε 5890); (0.1 N NaOH) 278 nm (ε 12,700), min 238 nm (ε 6300); nmr (D_2O) τ 2.15 (d, 1, J_{6-5} = 8.25 Hz, H_6), 3.79 (d, 1, J_{5-6} = 8.25 Hz, H_5), 4.01 (d, 1, $J_{1'-2'}$ = 4 Hz, $H_{1'}$), 6.46 (s, 3, 2′-OCH_3), 6.95 (s, 6, 4-$N(CH_3)_2$).

Anal. Calcd for $C_{12}H_{19}N_3O_5$: C, 50.52; H, 6.70; N, 14.73. Found: C, 50.21; H, 6.63; N, 14.63.

5-Chloro-2′-O-methyluridine (**11**). METHOD A. To a solution of 0.194 g (0.00075 mole) of 2′-*O*-methyluridine (**5**) in 2.5 ml of glacial acetic acid was added 1 ml of an 0.87 M solution of chlorine in carbon tetrachloride (Prystaš and Šorm,

1964). The solution was observed to rapidly decolorize and was allowed to stand at room temperature for 5 hr. The solution was evaporated to dryness and the residue was coevaporated with four 50-ml portions of 1:1 ethanol–toluene. This residue was stirred with 55 mg of sodium methoxide in 30 ml of methanol at room temperature for 2 hr to effect deacetylation. The solution was deionized by stirring with Dowex 50W-X8 (H^+) resin and filtered. The residue from the evaporated filtrate was triturated with 2 ml of absolute ethanol and cooled at 4°. A 0.22-g yield of crystals was obtained which was recrystallized from ethanol to give 0.20 g (90%) of pure **11**, mp 208–210°; $[\alpha]_D^{25}$ 18.9° (*c* 2, MeOH); uv (0.1 N HCl and H_2O) max 277 nm (ε 8570), min 239 nm (ε 1500); (0.1 N NaOH) max 275 nm (ε 6300), min 250 nm (ε 3300); nmr (D_2O) τ 1.67 (s, 1, H_6), 4.07 (d, 1, $J_{1'-2'}$ = 3.5 Hz, $H_{1'}$), 6.45 (s, 3, 2′-O-CH_3).

Anal. Calcd for $C_{10}H_{13}ClN_2O_6$: C, 41.03; H, 4.47; Cl, 12.12; N, 9.57. Found: C, 40,75; H, 4.10; Cl, 12.09; N, 9.93.

METHOD B. Using the above identical conditions and isolation procedure 0.27 g (0.001 mole) of **2** gave 0.2 g (69%) of 5-chloro-2′-*O*-methyluridine (**11**), mp 206–209°, identical with the product from method A.

5-Bromo-2′-O-methyluridine (**12**). METHOD A. A solution of 0.19 g (0.00075 mole) of 2′-*O*-methyluridine (**5**) in 5 ml of water was treated dropwise with bromine until a permanent yellow color persisted (Prystaš and Šorm, 1964). The solution was then evaporated to dryness and the residual syrup was triturated with 2 ml of absolute ethanol to give a colorless solid. This material was recrystallized from ethanol to give 0.23 g (90%) of 5-bromo-2′-*O*-methyluridine (**12**), mp 235–237°; $[\alpha]_D^{25}$ 4.36° (*c* 1, MeOH); uv (0.1 N HCl and H_2O) max 279 nm (ε 9900), min 242 nm (ε 1800); (0.1 N NaOH) max 278 nm (ε 7100), min 251 nm (ε 3800); nmr (Me_2SO-d_6) τ −1.78 (s, 1, 3-NH), 1.50 (s, 1, H_6), 4.19 (d, 1, $J_{1'-2'}$ = 4 Hz, $H_{1'}$), 5.16–5.6 (m, 2, 3′- and 5′-OH), 6.59 (s, 3, 2′-OCH_3).

Anal. Calcd for $C_{10}H_{13}BrN_2O_6$: C, 35.63; H, 3.88; Br, 23.71; N, 8.31. Found: C, 35.92; H, 3.60; Br, 24.05, N, 8.09.

METHOD B. The above procedure was used with 0.27 g (0.001 mole) of **2** to give 0.23 g (70.5%) of 5-bromo-2′-*O*-methyluridine (**12**), mp 235–237°, identical with the product from method A.

5-Iodo-2′-O-methyluridine (**13**). METHOD A. A mixture of 0.194 g (0.00075 mole) of 2′-*O*-methyluridine (**5**), 0.195 g (0.00075 mole) of iodine, 1.5 ml of chloroform, 18 ml of water, and 0.45 ml of 7 N nitric acid were mixed in that order and refluxed on the steam bath for 8 hr (Prusoff *et al.*, 1953). The resulting solution was cooled for 15 hr at 5° and 0.21 g (74%) of crystalline **13**, mp 230–235°, was collected by filtration. The product was recrystallized from ethyl alcohol to give needles: mp 242–245° dec; $[\alpha]_D^{25}$ −24.5° (*c* 1, dimethylformamide–H_2O, 1:1); uv (0.1 N HCl) max 288 nm (ε 7950), min 248 nm (ε 2200); (H_2O) max 288 nm (ε 7950), min 248 nm (ε 1900); (0.1 N NaOH) max 280 nm (ε 6000), min 251 nm (ε 3300); nmr (Me_2SO-d_6) τ −1.6 (s, 1, 3-NH), 1.52 (s, 1, H_6), 4.21 (d, 1, $J_{1'-2'}$ = 4 Hz, $H_{1'}$), 4.64–5.05 (m, 2, 3′- and 5′-OH), 6.61 (s, 3, 2′-OCH_3).

Anal. Calcd for $C_{10}H_{13}IN_2O_6$: C, 31.26; H, 3.41; I, 33.04; N, 7.29. Found: C, 31.29; H, 3.50; I, 33.40; N, 7.32.

METHOD B. The above procedure was followed using 0.272 g (0.001 mole) of **2**, 0.280 g (0.0011 mole) of iodine, **3** ml of chloroform, and 25 ml of water. Two crops of 5-iodo-2′-*O*-methyluridine (**13**) were obtained giving 0.265 g (69.5%) of crystalline **13**, mp 238–240° dec, identical with material from method A.

2′,3′-Di-O-methyluridine (**14**). A solution of 0.25 g (0.00087 mole) of 4-methoxy-1-(2,3-di-*O*-methyl-β-D-ribofuranosyl)-2-pyrimidinone (**4**) in **5** ml of water and 1 ml of methanol was stirred for 3 hr at room temperature with 2 ml of Dowex 50W-X8 (H^+) resin. The mixture was filtered and the resin was thoroughly washed with water. The combined filtrate was evaporated and the residue was recrystallized from ethanol to give 0.18 g (75%) of 2′,3′-di-*O*-methyluridine (**14**): mp 176–177°; $[\alpha]_D^{25}$ 74.7° (*c* 1.3, MeOH); uv (0.1 N HCl) max 261 nm (ε 11,200), min 230 nm (ε 2450); (H_2O) max 261 nm (ε 11,400), min 230 nm (ε 2450); (0.1 N NaOH), max 261 nm (ε 8400), min 242 nm (ε 5990); nmr (D_2O) τ 2.08 (d, 1, J_{6-5} = 8.25 Hz, H_6), 4.11 (d, 1, J_{5-6} = 8.25 Hz, H_5), 4.03 (d, 1, $J_{1'-2'}$ = 3.5 Hz, $H_{1'}$), 6.47 (s, 3, 2′-OCH_3), 6.53 (s, 3, 3′-OCH_3).

Anal. Calcd for $C_{11}H_{16}N_2O_6$: C, 48.52; H, 5.92; N, 10.29. Found: C, 48.40; H, 5.58; N, 10.11.

2′,3′-Di-O-methylcytidine (**15**). A solution of 0.29 g (0.001 mole) of **4** in 22 ml of 7% ammonia in methanol was heated in a sealed tube for 3 days at 100°. The solution was evaporated to dryness to give a white residue which was crystallized from benzene containing a few drops of ethanol. The product (0.22 g, 81%) had mp 72–75° when air-dried. This material was dried at 60–70° at 0.1 mm for 2 days to give crystals with mp 106–108°; $[\alpha]_D^{25}$ 112.6° (*c* 1, MeOH). The product absorbs water and other solvents as evident from nmr spectroscopy and could not be dried vigorously enough to reach a reliable melting point; uv (0.1 N HCl) max 278 nm (ε 13,000), min 240 nm (ε 1600); (H_2O) max 271 nm (ε 8810), min 250 nm (ε 6100); (0.1 N NaOH) max 271 nm (ε 9200), min 250 nm (ε 6500); nmr (D_2O) τ 2.10 (d, 1, J_{6-5} = 7.5 Hz, H_6), 3.93 (d, 1, J_{5-6} = 7.5 Hz, H_5), 4.05 (d, 1, $J_{1'-2'}$ = 2.5 Hz, $H_{1'}$), 6.43 (s, 3, 2′-OCH_3), 6.52 (s, 3, 3′-OCH_3).

Anal. Calcd for $C_{11}H_{17}N_3O_5$: C, 48.70; H, 6.32; N, 15.49. Found: C, 48.31; H, 6.77; N, 15.29.

N^4-Methyl-2′,3′-di-O-methylcytidine [*4-N-Methylamino-1-(2,3-di-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone*] (**16**). To a solution of 0.214 g (0.00075 mole) of **4** in 2 ml of methanol was added 10 ml of a cold 16% solution of methylamine in methanol. The solution was allowed to stand for 18 hr at room temperature and was evaporated to dryness. The residue was crystallized from ethyl acetate–ethanol (2:1, v/v) to give 0.162 g (76.5%) of **16**, mp 102–104°. These crystals were dried at 80–90° at 0.1 mm for 24 hr to give mp 167–168°; $[\alpha]_D^{25}$ 112.7° (*c*, 1.11, MeOH); uv (0.1 N HCl) max 281 nm (ε 14,600), min 242 nm (ε 2300); (H_2O) max 271 nm (ε 11,400), min 248 nm (ε 8100); (0.1 N NaOH) max 270 nm (ε 11,800), min 248 nm (ε 8400); nmr (D_2O) τ 2.24 (d, 1, J_{6-5} = 8 Hz, H_6), 4.0 (d, 1, J_{5-6} = 8 Hz, H_5), 3.99 (d, 1, $J_{1'-2'}$ = 3 Hz, $H_{1'}$), 6.41 (s, 3, 2′-OCH_3), 6.53 (s, 3, 3′-OCH_3), 7.10 (s, 3, 4-NCH_3).

Anal. Calcd for $C_{12}H_{19}N_3O_5$: C, 50.52; H, 6.70; N, 14.73. Found: C, 50.44; H, 6.62; N, 14.80.

N^4,N^4-Dimethyl-2′,3′-di-O-methylcytidine [*4-N,N-Dimethylamino-1-(2,3,-di-O-methyl-β-D-ribofuranosyl)-2-pyrimidinone*] (**17**). A solution of 0.214 g (0.00074 mole) of **4** in 4 ml of methanol was treated with a cold 45% solution of dimethylamine in methanol and the resulting solution was allowed to stand for 2 days at room temperature and then evaporated to dryness. The residue was triturated with 3 ml of ethanol to give 0.185 g (82%) of crystalline product, mp 135–137°. A sample of **17** was recrystallized from ethanol and dried at 80–90° at 0.1 mm; mp 143–145°; $[\alpha]_D^{25}$ 99.8° (*c* 1.6, MeOH); uv (0.1 N HCl) max 286 nm (ε 16,500), min 245 nm (ε 2500); (H_2O) max 275 nm (ε 14,400), min 239 nm (ε 6700); (0.1 N NaOH) max 275 nm (ε 14,700), min 239 nm (ε 7180); nmr (D_2O) τ 2.31 (d, 1, J_{6-5} = 8 Hz, H_6), 4.15 (d, 1, J_{5-6} = 8 Hz, H_5), 4.45 (d, 1, $J_{1'-2'}$ = 4 Hz, $H_{1'}$), 6.47 (s, 3, 2′-OCH_3), 6.56, (s, 3, 3′-OCH_3), 6.86 (s, 6, 4-$N(CH_3)_2$).

Anal. Calcd for $C_{13}H_{21}N_3O_5$: C, 52.16; H, 7.07; N, 14.04. Found: C, 51.86; H, 7.02; N, 13.90.

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