cer Research (USPHS Grants CA-06927 and RR-05539 and an appropriation from the Commonwealth of Pennsylvania).

Registry No.—**3** tetrasodium salt, 54677-85-9; **4** tetrasodium salt, 54677-86-0; **5**, 50304-49-9; **8a**, 54677-87-1; **8b**, 54677-88-2; **10a**, 54677-89-3; **10b**, 54677-90-6; **11a**, 54677-91-7; **11b**, 54724-59-3; **12**, 54677-92-8; **13**, 54677-93-9; **14a** disodium salt, 54677-94-0; **14a** ammonium salt, 54677-95-1; **14b** disodium salt, 54677-96-2; **15a** disodium salt, 54677-97-3; **15b** disodium salt, 54677-98-4; **16** disodium salt, 54677-99-5; **18a** sodium salt, 54678-00-1; **18b** sodium salt, 54678-01-2; **19a** disodium salt, 54678-02-3; 2′,3′-*O*-isopropylideneadenosine, 362-75-4.

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(21) Darco G-60 charcoal was used for adsorbing nucleotides and was activated prior to use by the method of Lipkin et al.[22] Petroleum ether employed in purifications boiled at 30–60°. Thin layer chromatograms were obtained with Merck F-254 silica gel plates in chloroform–methanol (9:1) (system A). Preparative layer chromatography was conducted with 2-mm layers of silica gel on glass. Paper chromatography (descending) employed Whatman No. 1, 3MM or 17 papers in (B) 2-propanol–concentrated NH_4OH–water (7:1:2); (C) 1-butanol–acetic acid–water (5:2:3); (D) 1-propanol–concentrated NH_4OH–water (7:1:2); (E) 1-propanol–concentrated NH_4OH–water (55:10:35); (F) isobutyric acid–1 *M* NH_4OH (10:6); (G) 1-butanol–acetic acid–water (4:1:5, upper layer); (H) 1-butanol–pyridine–water (3:1:1); (J) 1-butanol–pyridine (2:1). Electrophoresis was performed on Whatman No. 1 paper at 40–80 V/cm for 30–60 min at pH 7.5 [0.05 *M* $(Et)_3NHCO_3$, pH 4.5 (0.05 *M* acetate), or pH 3.5 (0.015 *M* citrate)]. Mobility values (M_{AMP}) are relative to those of adenosine 5′-phosphate (AMP). Spots on chromatograms were detected by their ultraviolet absorption and (in the case of silica gel chromatograms) by spraying with the Molisch reagent. Melting points (uncorrected) were determined by the capillary method. Ultraviolet spectra were determined with a Cary Model 15 spectrophotometer. Infrared spectra were determined in KBr disks with a Perkin-Elmer spectrophotometer Model 137, and ^1H NMR spectra were obtained with a Varian XL-100-15 spectrometer and are recorded as parts per million downfield from an external standard (concentric capillary) of $SiMe_4$. Infrared and NMR spectra of nucleotides were usually obtained on ammonium salts from which the analytically pure sodium salts were obtained. Specific rotations were determined with a Bendix automatic polarimeter 1169. Elemental analyses were performed by Atlantic Microlabs, Atlanta, Ga., and Midwest Microlab, Ltd., Indianapolis, Ind.
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Nucleotides. V. Syntheses of 2′-*O*- and 3′-*O*-(3-Methyl-2-picolyl 1-oxide) Ribonucleosides and Diribonucleoside Monophosphates by Application of 3-Methyl-2-picolyl 1-Oxide Protection[1]

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The alkylation of ribonucleosides (uridine series, **3** and **6**; adenosine series, **16** and **19**) with 1-oxido-3-methyl-2-pyridyldiazomethane (**2**) afforded an isomeric mixture of 2′-*O*- and 3′-*O*-(3-methyl-2-picolyl 1-oxide) ribonucleosides in 63–91% yields. 2′-*O*-(3-Methyl-2-picolyl 1-oxide)uridine (**4**) and 2′-*O*- (**17**), and 3′-*O*-(3-methyl-2-picolyl 1-oxide)adenosine (**18**) could be isolated by fractional crystallization of the respective isomeric mixture, whereas 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-benzoyluridine (**7**) and 2′-*O*-(3-methyl-2-picolyl 1-oxide)-$O^{5'}$,N^6-dibenzoyladenosine (**20**) were isolated by column chromatography on silica gel. The stability of 3-methyl-2-picolyl 1-oxide group toward tritylation, benzoylation, and especially phosphorylation by the general method (phosphate in the presence of TPS) and its removability were found to be compatible with the oligoribonucleotide synthesis. Thus, the synthesis of 2′-*O*-(3-methyl-2-picolyl 1-oxide)uridylyl(3′–5′)-$O^{2'}$,$O^{3'}$,N^6-triacetyladenosine (**26**) and uridylyl(3′–5′)adenosine (UpA, **27**) was achieved by the application of 3-methyl-2-picolyl protection.

Requirements for protecting groups in the oligoribonucleotide synthesis have been recently discussed by Christen and Broom.[2] The development of the synthesis by a "phosphotriester approach",[3] in particular, has depended to a significant extent on the design of a new protecting group for 2′-hydroxyl function which meets the requirements.[4] Since 2′-*O*-(2-picolyl 1-oxide) ribonucleoside might be useful key intermediates for the oligonucleotide synthesis, our interest is selective and direct introduction of a removable blocking group of this type into the *cis*-glycol system of ribonucleosides. We have therefore prepared a series of nitrogenous heterocyclic *N*-oxides bearing a diazomethylene group and it was concluded that out of these diazoalkanes, 1-oxido-3-methyl-2-pyridyldiazomethane (**2**), might be a reagent of choice for the monoalkylation of the *cis*-glycol system of the ribonucleosides because of its easy accessibility and comparatively small δ value of the signal due to the diazomethylene proton in its NMR spectrum.[5]

Table I
NMR Data[a] of 2′-*O*- and 3′-*O*-(3-Methyl-2-picolyl 1-oxide) Nucleosides

O-(3-Methyl-2-picolyl 1-oxide) of	Anomeric proton		3″-Methyl proton	
	2′-*O*-	3′-*O*-	2′-*O*-	3′-*O*-
Uridine[c]	5.93	5.77	2.37	2.44
Adenosine	5.76	5.59	2.30	2.50
5′-*O*-Benzoyluridine	5.90	5.67	2.46	2.42
$O^{5'}$,N^{6}-Dibenzoyl-adenosine	6.16	6.11	2.37	2.45

[a] In $CDCl_3$, in parts per million (δ). [b] Reference 6. [c] In DMSO-d_6.

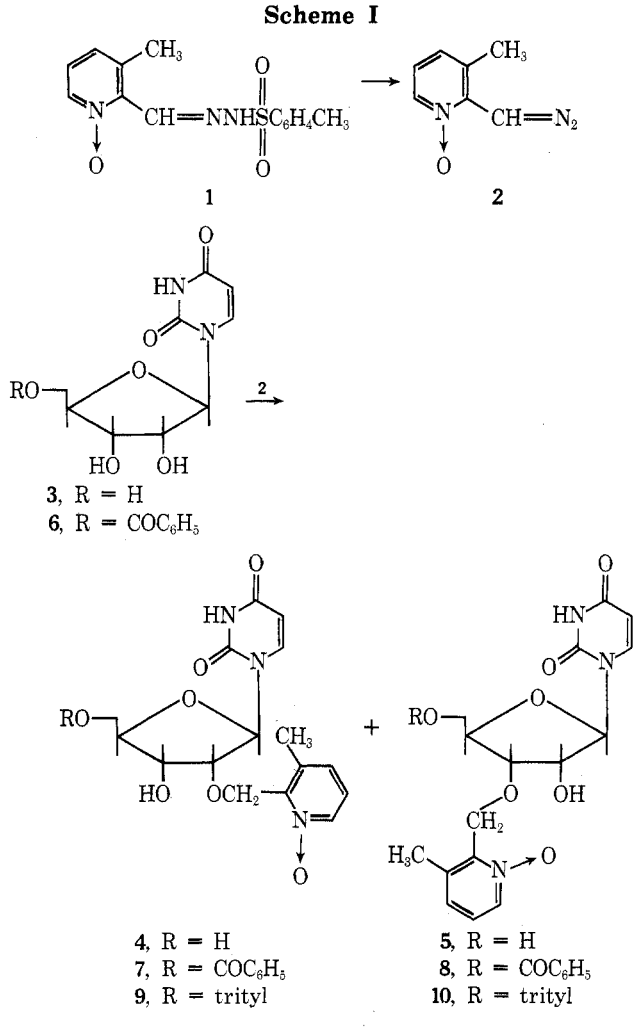
The present paper deals with the synthesis of 2′-*O*- and 3′-*O*-(3-methyl-2-picolyl 1-oxide) ribonucleosides (uridine series, **4–10**; adenosine series, **17–23**) by the use of **2** and the synthesis of uridylyl(3′–5′)adenosine (UpA) by the application of 1-oxido-3-methyl-2-picolyl protection.

Alkylation of uridine (**3**) with freshly prepared **2** in the presence of $SnCl_2$ (Scheme I) was performed essentially according to Christensen and Broom's procedure.[2] The reaction took place smoothly with evolution of nitrogen gas and within 12 hr the starting nucleoside (**3**) disappeared in the reaction mixture. After work-up, a 1:1 mixture of 2′-*O*- (**4**) and 3′-*O*-(3-methyl-2-picolyl 1-oxide)uridine (**5**) was obtained as a solid in combined yield of 91%. Recrystallization of this isomeric mixture from water afforded pure 2′-O isomer (**4**) in 25% yield. Attempted chromatography (silica gel) of the mother liquor failed to separate 3′-*O*-(3-methyl-2-picolyl 1-oxide)uridine (**5**) from **4**. The mixture was therefore treated with trityl chloride in pyridine to give monotritylated derivatives (**9** and **10**) from which 3′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (**10**) was isolated in 39% yield by column chromatography. A pure sample of **5** was prepared by detritylation of **10** in 90% yield. The purity of **4** and **5** could be readily demonstrated by examination of the signals, particularly by examination of the signals for 3″-CH_3[6] which appeared as a three-proton singlet at 2.37 and 2.44 ppm, respectively (see Table I). Structural assignments of **4** and **5** rest upon elemental and spectral (uv and NMR) analysis. As pointed out earlier by Reese and coworkers,[7] among a pair of 2′-O and 3′-O isomers, NMR signals due to the anomeric proton of the former all appeared at lower field (Table I). The fact that uridylyl(3′–5′)adenosine prepared starting from **4** was completely hydrolyzable with bovine pancreatic RNase to uridine 3′-phosphate and adenosine (vide infra) confirmed the structural assignment of **4** and hence **5**.

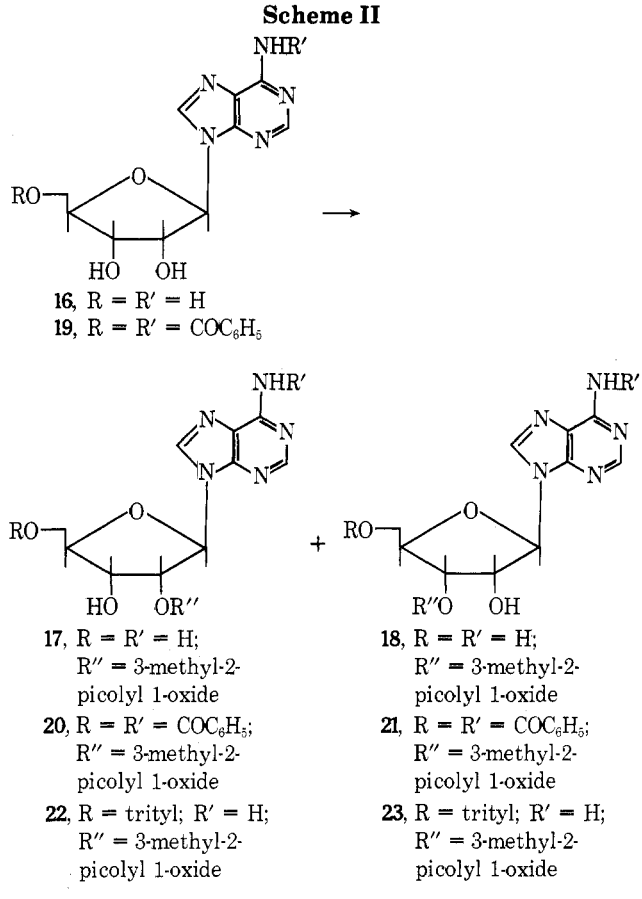
Parallel experiments with adenosine (**16**) and **2** afforded a 1:1 mixture of 2′-*O*- (**17**) and 3′-*O*-(3-methyl-2-picolyl 1-oxide)adenosine (**18**) in 90% yield (Scheme II). Separation of each isomer was achieved by taking advantage of a large difference in their methanol solubility. Thus, on trituration of this isomeric mixture with methanol, a large proportion of 3′-O isomer (**18**) was dissolved in the solvent, 2′-O isomer (**17**) remaining undissolved. Recrystallization of the latter (**17**) from water afforded, after evaporation of the solvent followed by crystallization from ethanol, a pure sample of 3′-O isomer (**18**) in 13% yield. These structural assignments again rest upon both elemental and spectral (uv and NMR) analysis. NMR spectral trends follow the uridine case (Table I).

It is worthy of note that the solubility both in water and in methanol was remarkably different between the 2′-O

Scheme I



Scheme II



and 3′-O isomers in the adenosine series as well as the uridine series. Thus the 3′-O isomers (**5** and **18**) are nearly freely soluble in water whereas the 2′-O isomers (**4** and **17**) have a low solubility in water, which may serve as a suitable solvent for recrystallization of the latter.

In addition to the above studies with the free nucleosides (**3** and **16**), the alkylation of 5′-*O*-benzoyluridine (**6**) and $O^{5'},N^6$-dibenzoyladenosine (**19**) was also undertaken. As far as we know, the nucleoside **6** has never been described in the literature and **6** was therefore prepared in the following way. Benzoylation of 2′,3′-*O*-isopropylideneuridine afforded the corresponding 5′-*O*-benzoyl derivative, which in turn was treated with 80% aqueous acetic acid (100°, 4 hr) to afford the required **6** in 54% overall yield. The structure was confirmed by both combustion values and NMR analysis. The nucleoside **6** was treated with freshly prepared **2** as in the case of uridine. Evaporation of the solvent left crude products (**7** and **8**) contaminated with by-products. Attempted fractional recrystallization failed because of the slight difference in the solubility in water between two isomers. The mixture was therefore separated with the aid of silica gel chromatography. The yields of 2′-*O*- (**7**) and 3′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-benzoyluridine (**8**) were 34.4 and 18.7%, respectively. These structural assignments rest upon the fact that among a pair of isomers, an NMR signal due to the anomeric proton **7** appeared at relatively lower field (see Table I).[7] It is worthy of note, however, that the signal due to the 3″-methyl proton[6] of the 3′-O isomer (**8**) appeared upfield in the NMR spectrum relative to that of the 2′-O isomer (**7**) and this contrasts with other pairs of isomers (see Table I) where the signals due to 3″-methyl protons of the 2′-O isomers appeared relatively upfield.

$O^{5'},N^6$-Dibenzoyladenosine (**19**), which was prepared from 2′,3′-*O*-ethoxymethylene-$O^{5'},N^6$-dibenzoyladenosine by acid hydrolysis was treated with **2** to afford a mixture of 2′-*O*- (**20**) and 3′-*O*-(3-methyl-2-picolyl 1-oxide)-$O^{5'},N^6$-dibenzoyladenosine (**21**). The relative yields of two isomers were determined as roughly 1:1 by relative areas of the 3″-methyl[6] absorption in the NMR spectrum. Since once again the solubility difference in both water and methanol between these isomers was not large enough to permit the fractional recrystallization, the mixture was subjected to chromatographic (silica gel) separation. The compound (**20**) was isolated pure in 25% yield and the structure was determined on the basis of elemental analysis and Reese's rule.[7] However, even by this chromatographic technique we failed to obtain pure **21**.

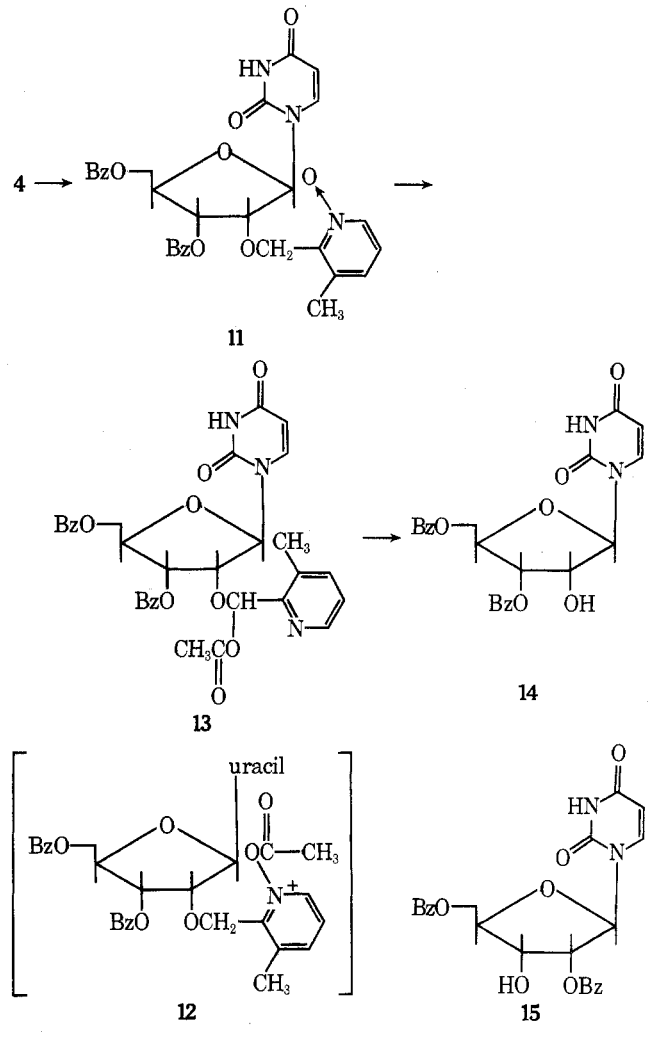
Alkylation of 5′-*O*-trityluridine[8] with **2** in the presence of $SnCl_2$ was found to be accompanied by detritylation to give an isomeric mixture of **4** and **5**.

In the connection, with the catalyst $SnCl_2$ it must be emphasized that the alkylation in the presence of an excess of the catalyst afforded a substantial amount of uv-absorbing by-product(s) of unknown structure[9] and the yield of required products was accordingly reduced to a significant extent.

Our next objective was to examine the stability of the 3-methyl-2-picolyl 1-oxide group toward a variety of reagents encountered in the nucleotide synthesis: tritylation, benzoylation, and phosphorylation [in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPS)],[10] and its removability under required conditions.

Tritylation of 2′-*O*-(3-methyl-2-picolyl 1-oxide)uridine (**4**) by a conventional method afforded the corresponding 5′-*O*-trityl derivative (**9**) in almost quantitative yield.[8b] Benzoylation of **4** with benzoyl chloride (1.86 equiv) afforded 3′,5′-di-*O*-benzoyl-2′-*O*-(3-methyl-2-picolyl 1-oxide)uridine (**11**) in 58% yield (Scheme III). The structural assignment rests upon the combustion values and spectral data.

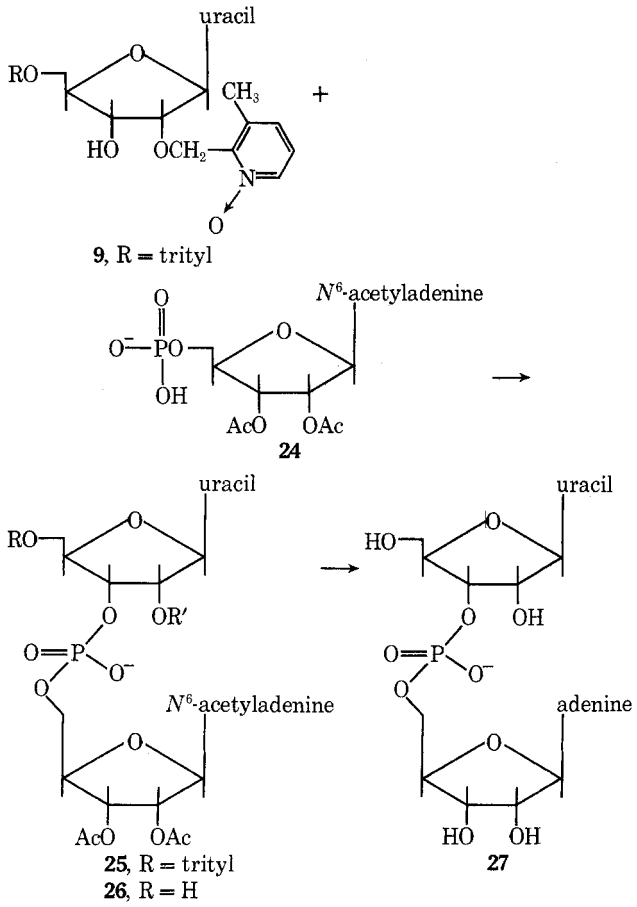
Scheme III



A deblocking experiment was carried out taking **11** as an example in the following way. The nucleoside **11** was treated with acetic anhydride at 43°. The progress of the reaction was monitored by TLC. Shortly (10 min) after the start of the reaction, the formation of an intermediate (presumably *N*-acetoxypyridinium acetate derivative, **12**)[11] was observed on TLC. After 14 hr, the formation of 3′,5′-di-*O*-benzoyl-2′-*O*-(3-methyl-2-pyridylacetoxymethyl)uridine (**13**) was observed. After 6 days both **11** and **12** completely disappeared in the reaction mixture, after which time the reaction mixture was worked up and a product (**13**) was isolated pure by silica gel chromatography. Although we failed to crystallize this nucleoside because of the epimeric mixture, it was found to be homogeneous on the criteria of chromatographic behavior and NMR spectra. Its combustion values were also compatible with the structure assigned. The yield of **13** was quantitative. Acidic hydrolysis (50% aqueous acetic acid, 70°, 3 hr) converted **13** into crystalline 3′,5′-di-*O*-benzoyluridine (**14**) in almost quantitative yield. Spectral (NMR) analysis showed that this was indeed 3′,5′-di-*O*-benzoyluridine and not 2′,5′-*O*-benzoyluridine.[12]

The remainder of this section deals with the synthesis of uridylyl(3′–5′)adenosine (UpA, **27**). Treatment of 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (**9**) with $O^{2'},O^{3'},N^{6'}$-triacetyladenosine 5′-phosphate (**24**) in the presence of TPS (Scheme IV) afforded 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityluridylyl(3′–5′)-$O^{2'},O^{3'},N^6$-tria-

Scheme IV



cetyladenosine (**25**). Acidic treatment of **25** under mild conditions afforded the corresponding dinucleoside monophosphate derivative (**26**), which might be of use for further chain elongation. For the complete deblocking the nucleoside **26** was first treated with acetic anhydride (at 43° for 6 days) and then with methanolic ammonia (at room temperature) to give **27** in 92% yield. As already mentioned, this sample of **27** was found to be completely hydrolyzable with pancreatic RNase[13] to give uridine 3′-phosphate and adenosine in 1:1 molar ratio.[14]

Thus ease of introduction, stability, and removability under required conditions of the 3-methyl-2-picolyl 1-oxide group was found to be completely compatible with the oligonucleotide synthesis. Our approach by the application of this novel 2′-O-protecting group might have a considerable merit in the oligonucleotide synthesis starting from synthetic modified nucleosides (e.g., 1- or 3-deazaadenosine). The synthesis of oligoribonucleotides containirg the modified nucleosides as well as cytidine and guanosine and the oligonucleotides of higher chain length is now underway in our laboratory.

Experimental Section

General. Melting points were taken on a Yamato MP-1 capillary apparatus and are uncorrected. Ultraviolet absorption (uv) spectra were determined on a Hitachi spectrophotometer, Type 14. Infrared (ir) spectra were determined on a Model DS-701G spectrometer (Nippon Bunko Co.). Nuclear magnetic resonance (NMR) spectra were recorded on a Hitachi high-resolution NMR spectrometer, Model R24, and NMR signals are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; bs, broad singlet; bd, broad doublet. The chemical shifts were reported in parts per million downfield from Me_4Si (an internal standard). Elemental analyses were performed by a staff of the analytical room, Faculty of Pharmaceutical Sciences, Hokkaido University. Thin layer chromatography (TLC) was run on glass plates coated with silicic acid. Paper electrophoresis was performed on Toyo Roshi filter paper No. 51A at pH 7.5 using 0.05 *M* triethylammonium bicarbonate (TEAB) solution (20 V/cm, 1 hr). DEAE cellulose was a product of Jujo Paper Co. and a gift therefrom. RNase was obtained from Worthington Biochemicals. Digestion with this enzyme was performed as reported.[15] Unless otherwise stated, solvent was removed under reduced (aspirator) pressure with a rotating evaporator.

2′-*O*-(3-Methyl-2-picolyl 1-oxide)uridine (4) and an Isomeric Mixture of 4 and 3′-*O*-(3-Methyl-2-picolyl 1-oxide)uridine (5). To a stirred solution of uridine (**3**, 5.2 g, 21 mmol) and $SnCl_2 \cdot 2H_2O$ (100 mg) in DMF (50 ml) was added in three portions a DMF solution (1 ml) of 1-oxido-3-methyl-2-pyridyldiazomethane (**2**)[5] prepared from 16.49 g (54 mmol) of 3-methyl-2-formylpyridine 1-oxide *p*-tosylhydrazone (**1**). After 2 hr, a further amount of $SnCl_2 \cdot 2H_2O$ (100 mg) was added. The stirring was continued at room temperature overnight. After it was ascertained by TLC that the reaction was almost complete, the solvent was evaporated to leave an oily residue which was triturated with ethanol to precipitate a solid which was collected by filtration and dried, yield 7.0 g (91%). Recrystallization from water afforded **4**, yield 1.9 g (25%). This sample was found to be completely free of the 3′-O isomer (**5**) on the criterion of the NMR spectra: NMR (DMSO-d_6) δ 8.20 (q, J = 7.5 Hz, 1, H-6″ [6]), 7.92 (d, J = 10 Hz, 1, H-6), 5.93 (d, J = 7.0 Hz, 1, H-1′), 5.65 (bd, J = 10 Hz, 1, H-5), 4.9 (s, 2, H-7″), 2.37 (s, 3, 3″-CH_3); mp 266–268° dec.

Anal. Calcd for $C_{16}H_{19}N_3O_7 \cdot \frac{1}{2}H_2O$: C, 51.47; H, 5.09; N, 11.26. Found: C, 51.70; H, 5.30; N, 11.32.

3′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (10). To the above isomeric mixture (**4** and **5**, 3.05 g, 8.35 mmol) in pyridine (30 ml) was added with stirring trityl chloride (3 g, 11 mmol). The stirring was continued at room temperature until the starting materials were almost completely consumed (for 2 days). Evaporation of the solvent left a foam which was applied to a column (silica gel, 90 g). The column was washed with $CHCl_3$–EtOH (25:1). The eluate was monitored by TLC. The faster travelling fraction afforded, after removal of the solvent, a mixture of 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (**9**) and the 3′-O isomer (**10**) (0.8 g) and slower travelling fraction (free from **9**) was pooled, and concentrated to dryness. The residue was crystallized from methanol to give **10**: mp 246–248°; yield 2.0 g (39%); NMR ($CDCl_3$) δ 8.86 (d, $J_{5,6}$ = 10 Hz, 1, H-6), 8.25 (bt, 1, H-6″ [6]), 5.96 (bs, 1, H-1′), 5.40 (d, $J_{5,6}$ = 10 Hz, H-5), 2.37 (s, 3, 3″-CH_3[6]).

Anal. Calcd for $C_{35}H_{33}N_3O_7$: C, 69.19; H, 5.43; N, 6.91. Found: C, 69.25; H, 5.40; N, 6.83.

3′-*O*-(3-Methyl-2-picolyl 1-oxide)uridine (5). 3′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (**10**, 500 mg) was dissolved in 25 ml of aqueous acetic acid [AcOH–H_2O (20:5)]. The solution was allowed to stand at room temperature for 2 days, during which time triphenylmethyl alcohol (160 mg) precipitated and filtered off. The filtrate was concentrated to dryness. The residue was recrystallized from methanol: mp 216–218°; yield 270 mg (90%); NMR (DMSO-d_6) δ 5.77 (d, 4.0 Hz, 1, H-1′), 2.44 (s, 3, 3″-CH_3[6]).

Anal. Calcd for $C_{16}H_{19}N_3O_7$: C, 52.60; H, 5.20; N, 11.51. Found: C, 52.30; H, 5.15; N, 11.25.

2′-*O*-(3-Methyl-2-picolyl 1-oxide)-3′,5′-di-*O*-benzoyluridine (11). To a stirred suspension of **4** (1.65 g, 4.4 mmol) in pyridine (30 ml) was added in portions benzoyl chloride (1.14 g, 8.1 mmol) at 0°. The stirring was continued at room temperature overnight. Evaporation of the solvent left a solid which was purified by column chromatography [silica gel, solvent system $CHCl_3$–EtOH (25:1)], yield 1.5 g (58%). Recrystallization from methanol afforded an analytical sample: mp 129–131°; NMR ($CDCl_3$) δ 6.61 (d, J = 6 Hz, 1, H-1′), 2.18 (s, 3, 3″-CH_3[6]).

Anal. Calcd for $C_{30}H_{27}N_3O_9 \cdot \frac{1}{2}H_2O$: C, 61.85; H, 8.84; N, 7.21. Found: C, 61.80; H, 4.88; N, 7.13.

3′,5′-Di-*O*-benzoyluridine (14). Conversion of 11 into 14 via 13. A solution of **11** (800 mg, 1.37 mmol) in acetic anhydride (30 ml) was allowed to stand at 43° (bath temperature). The progress of the reaction was followed by TLC [solvent system $CHCl_3$–EtOH (7:1)]. In the very early stage of reaction (10 min) a new spot appeared on TLC which was assumed to correspond to *N*-acetoxypyridinium salt (**12**),[11] but it was not confirmed. After 14 hr another new spot due to **13** began to appear, with concomitant decrease in the area due to **12**. The reaction was considered to be complete when the spot due to **12** was scarcely discernible on TLC. It took 6 days. The mixture was then concentrated to dryness and the residue was applied to a silica gel column. The column was washed with $CHCl_3$–EtOH (33:1). The fraction containing 3′,5′-di-*O*-ben-

zoyl-2′-*O*-(3-methylpyridylacetoxymethyl)uridine (**13**) was collected. Evaporation of the solvent left a colorless and homogeneous foam, yield 845 mg (97%), which was treated with 80% aqueous acid (100 ml) at 70° for 3 hr. The cooled solution was concentrated to dryness. The residue was crystallized from methanol or aqueous methanol to afford an analytical sample of **14**, mp 187–189°, yield 535 mg (quantitative).

Anal. Calcd for $C_{23}H_{20}N_2O_8$: C, 61.06; H, 4.42; N, 6.19. Found: C, 61.02; H, 4.32; N, 6.25.

2′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (9). To a solution of 2′-*O*-(3-methyl-2-picolyl 1-oxide)uridine (**4**, 3.0 g, 8 mmol) in pyridine (50 ml) was added with stirring trityl chloride (3.4 g, 12 mmol) at 0°. The stirring was continued at 36° for 4 days. After work-up, the product was purified by column chromatography [silica gel, 90 g; solvent system $CHCl_3$–EtOH (25:1)]. Evaporation of the fraction containing **9** left a homogeneous foam, yield 4.5 g (92%).

Anal. Calcd for $C_{35}H_{33}N_3O_7$: C, 69.17; H, 5.47; N, 6.91. Found: C, 69.22; H, 5.56; N, 7.01.

5′-*O*-Benzoyluridine (6). To a cooled solution of 2′,3′-*O*-isopropylideneuridine (5.7 g, 20 mmol) in pyridine (20 ml) was added with stirring a pyridine solution (10 ml) of benzoyl chloride (3.1 g, 22 mmol) over a period of 30 min. The stirring was continued at room temperature overnight. The mixture was then concentrated to dryness and the residue was dissolved in chloroform (100 ml). The solution was successively washed with water and 5% sodium hydrogen carbonate solution, and dried (Na_2SO_4). The salt was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in 80% acetic acid (100 ml) and the solution was heated at 100° for 4 hr. The solvent was removed to give a product which was dried by codistillation with ethanol. The final residue was dissolved in ethanol (50 ml). There was then added dry ether (150 ml) to give crystalline (homogeneous) product (6.0 g). Recrystallization from ethanol or chloroform afforded an analytical sample, mp 169–170°, yield 4.4 g (63%).

Anal. Calcd for $C_{16}H_{16}N_2O_7$: C, 55.17; H, 4.60; N, 8.05. Found: C, 55.21; H, 4.60; N, 7.99.

2′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-benzoyluridine (7) and 3′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-benzoyluridine (8). To a stirred suspension of 5′-*O*-benzoyluridine (**6**, 2.0 g, 5.74 mmol) and $SnCl_2 \cdot 2H_2O$ (100 mg) in DMF (20 ml) was added a chloroform solution (1 ml) of **2**,[5] prepared from **1** (4.4 g, 14.5 mmol) and sodium (322 mg, 14 mmol). The stirring was continued at room temperature overnight, during which time a clear solution resulted. After work-up, the crude products were dissolved in methanol (100 ml) and the insoluble material (900 mg)[9] was filtered off. Evaporation of the filtrate left crude products which were dissolved in chloroform and applied to a silica gel column (silica gel, 100 g). The column was washed with $CHCl_3$–EtOH (25:1). The fraction containing 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-benzoyluridine (**7**) was collected. Evaporation of the solvent left **7** as a homogeneous foam. From the subsequent fraction an almost pure sample of **7** (820 mg) was obtained, which was rechromatographed over silica gel with the same solvent. Evaporation of the solvent afforded a further crop of **7** as a homogeneous foam, combined yield 1.0 g (34.4%). On the basis of the δ value of the signal due to the anomeric proton (5.90 ppm),[7] compound **7** was assigned as the 2′-O isomer. The third fraction afforded after evaporation of the solvent a mixture of 3′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-benzoyluridine (**8**) and **7**, yield 200 mg. The fourth fraction afforded on similar treatment a pure sample of **8** (500 mg, 18.7%): NMR ($CDCl_3$) δ 5.67 (d, J = 1 Hz, 1, H-1′), 2.42 (s, 3, 3″-CH_3[6]). On the basis of the δ value, compound **8** was assigned as the 3′-O isomer.

Anal. Calcd for $C_{23}H_{23}N_3O_8$: C, 55.84; H, 4.90; N, 8.95. Found: C, 58.69; H, 4.73; N, 8.88.

2′-*O*- (17) and 3′-*O*-(3-Methyl-2-picolyl 1-oxide)adenosine (18). A mixture of adenosine (**16**, 10 g, 37.8 mmol) and $SnCl_2 \cdot 2H_2O$ (100 mg) in DMF (200 ml) was heated until a clear solution resulted. To the cooled solution was added at room temperature a chloroform solution (1 ml) of **2**, freshly prepared from **1** (19.02 g, 62 mmol, 2 equiv). After 18 hr the solvent was evaporated to leave a product (crude yield of **17** and **18**, 90%) which was triturated with hot ethanol (150 ml). The insoluble material was collected by filtration and washed with methanol to give **17** (6.0 g, 41%). Recrystallization from water afforded an analytical sample of **17** (5.0 g, 34.1%). This compound was tentatively assigned as the 2′-O isomer on the basis of δ values of the anomeric proton (5.76 ppm) and 3″-methyl protons[6] (2.30 ppm).

Anal. Calcd for $C_{17}H_{20}N_6O_5$: C, 52.24; H, 5.24; N, 21.67. Found: C, 52.24; H, 5.24; N, 21.67.

The above filtrate (ethanol solution) deposited a mixture of **17** and **18** (7.0 g) after standing at room temperature for 3 days. The mixture was collected by filtration and triturated with methanol (50 ml) to give a further crop of **17** (500 mg, 3.3%) as the insoluble fraction. Concentration of the mother liquor left oily residue which was triturated with ethanol and the insoluble fraction was collected by filtration and recrystallized from ethanol to afford an analytical sample of **18**, yield 2.0 g(13%). This sample was found to be very hygroscopic. Compound **18** was assigned as the 3′-O isomer on the basis of δ values of the signals due to the anomeric proton (5.59 ppm) as well as 3″-methyl protons[6] (see Table I).

Anal. Calcd for $C_{17}H_{20}N_6O_5$: C, 52.53; H, 5.24; N, 21.67. Found: C, 52.44; H, 5.24; N, 21.78.

***O*⁵′,*N*⁶-Dibenzoyladenosine (19).** To a stirred suspension of adenosine (**16**, 10.0 g, 37.6 mmol) in ethyl orthoformate (40 ml) was added *p*-toluenesulfonic acid (monohydrate, 8.0 g). The stirred suspension was refluxed for 45 min, during which period a clear solution resulted. The cooled solution was neutralized with 80 ml of 0.4 *M* methanolic sodium methoxide. The sodium tosylate which precipitated was removed by filtration. The filtrate was concentrated to dryness, the residue was dissolved in chloroform (200 ml), and the insoluble material was filtered off. The residue, obtained by evaporation of the filtrate, was purified by silica gel chromatography; the yield of 2′,3′-*O*-ethoxymethylideneadenosine was 10.0 g. The adenosine derivative was dissolved in pyridine (100 ml) and treated with benzoyl chloride (20 g) at room temperature overnight. The mixture was concentrated to a half of its volume and then poured into a saturated sodium carbonate solution (150 ml) at around 5°. The product was extracted with three 150-ml portions of chloroform. The organic layer was washed with water and dried over sodium sulfate. The inorganic salt was filtered off and the filtrate was concentrated to dryness. The residue (crude 2′,3′-*O*-ethoxymethylidene-*O*⁵′,*N*⁶-dibenzoyladenosine) was dissolved in a mixture of acetic acid (80 ml) and water (200 ml). The solution was allowed to stand at room temperature overnight and then concentrated to dryness. The residue was partitioned between chloroform (50 ml) and saturated sodium carbonate solution (20 ml). The organic layer was separated and dried over sodium sulfate. The inorganic salt was filtered off and the filtrate was concentrated to dryness. The residue was applied to a column [silica gel, 200 g; solvent system $CHCl_3$–EtOH (100:3)]. The fraction containing the required product (**19**) was pooled and concentrated to give a colorless and homogeneous foam (8.4 g, 45%). This sample showed the positive test toward the *cis*-glycol–metaperiodate–benzidine test.[16] The structure was also confirmed by spectral (uv and NMR) as well as combustion analyses, uv (EtOH) λ_{max} 230, 279 nm,[17] NMR spectra showing the presence of ten aromatic protons (dibenzoyl).

Anal. Calcd for $C_{24}H_{21}N_5O_6H_2O$: C, 58.41; H, 4.66; N, 14.19. Found: C, 58.08; H, 4.25; N, 14.26.

2′-*O*-(3-Methyl-2-picolyl 1-oxide)-*O*⁵′,*N*⁶-dibenzoyladenosine (20) and 3′-*O*-(3-methyl-2-picolyl 1-oxide)-*O*⁵′,*N*⁶-dibenzoyladenosine (21). To a stirred solution of **19** (2.0 g, 4.21 mmol) and $SnCl_2 \cdot 2H_2O$ (20 mg) in DMF (30 ml) was added in portions a chloroform solution (1 ml) of **2** which was freshly prepared from **1** (2.0 g, 7.6 mmol) and sodium (147.4 mg) in ethanol (30 ml). The stirring was continued at room temperature for 8 hr. The mixture was then concentrated to dryness. The residue was applied to a silica gel column (silica gel, 100 g). The column was washed with $CHCl_3$–EtOH (25:1). The fraction containing **20** was collected. Evaporation of the solvent left a homogeneous foam (100 mg): uv (EtOH λ_{max} 230, 279 nm; NMR ($CDCl_3$) δ 8.70 (s, 1, H-8 or H-2), 8.21 (s, 1, H-2 or H-8), 6.16 (d, $J_{1',2'}$ *1, H-1′*), = 7 Hz, 2.37 (s, 3, 3″-CH_3[6]). On the basis of δ values of the anomeric proton and 3″-methyl,[6] compound **20** was assigned the 2′-O isomer (see Table I).

Anal. Calcd for $C_{31}H_{28}N_6O_7 \cdot \frac{1}{2}H_2O$:C, 61.48; H, 4.79; N, 13.88. Found: C, 61.78; H, 4.80; N, 13.63.

The subsequent fraction afforded after evaporation of the solvent a mixture of **20** and **21**, yield 3.02 g, uv (EtOH) λ_{max} 230, 279 nm. Since every signal in the NMR spectra of **20** had been assigned (vide ante), signals of **21** could be assigned from the NMR spectra of the mixture as follows: NMR ($CDCl_3$) δ 6.05 (d, J = 1 Hz, 1, H-1′), 2.30 (s, 3, 3″-CH_3[6]).

3′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-trityladenosine (23). To a solution of 3′-*O*-(3-methyl-2-picolyl 1-oxide)adenosine (**18**, 500 mg, 1.286 mmol) in pyridine (30 ml) was added trityl chloride (700 mg, 2.51 mmol). The mixture was stirred for 4 days at room temperature and then concentrated to dryness. The residue was dissolved in a mixture of methanol (20 ml) and saturated sodium carbonate solution (50 ml). The resulting solution was extract-

ed with four 30-ml portions of chloroform. The combined chloroform solution was dried (Na_2SO_4) and filtered. The filtrate was concentrated to dryness. The residue was applied to a column silica gel, 11 g). The column was washed with $CHCl_3$–EtOH (25:1). The eluate was monitored by TLC [solvent system $CHCl_3$–EtOH (7:1)]. The fraction containing **23** was pooled and concentrated to dryness (homogeneous foam): yield 520 mg (76%); NMR ($CDCl_3$) δ 8.27 (s, 1, H-8 or H-2), 8.04 (s, 1, H-2 or H-8), 6.05 (d, J = 4.9 Hz, 1, H-1'), 2.36 (s, 3, 3''-CH_3[6]).

Anal. Calcd for $C_{36}H_{34}N_6O_5 \cdot \frac{3}{2}H_2O$: C, 65.75; H, 5.63; N, 12.78. Found: C, 65.87; H, 5.24; N, 12.64.

Uridylyl(3'–5')adenosine (27). A stock solution[18] of $O^{2'},O^{3'},N^6$-triacetyladenosine 5'-phosphate (8.3 ml) was dissolved in dry pyridine (15 ml). The resulting mixture was concentrated to dryness in vacuo. This process was repeated four times. The final residue and 2'-*O*-(3-methyl-2-picolyl 1-oxide)-5'-*O*-trityluridine (**9**, 141.2 mg, 0.23 mmol) were dissolved in pyridine (15 ml). The mixture was then three times codistilled with dry pyridine (3 × 15 ml). The final residue was dissolved in pyridine (30 ml) containing triisopropylbenzenesulfonyl chloride (1.035 g, 0.345 mmol). The solution was kept at room temperature overnight. Water (1 ml) was then added and the solution was concentrated to dryness in vacuo.

Detritylation. The above residue was dissolved in acetic acid (40 ml) and water (20 ml). The mixture was allowed to stand at 45° overnight. After it was ascertained by paper electrophoresis (0.05 *M* TEAB solution, pH 7.5, 20 V/cm, 1.5 hr) that detritylation was complete, the mixture was concentrated to dryness. The residue was applied to DEAE cellulose column (column size 15 × 3 cm). Elution was performed by a linear gradient of 0.02 *M* TEAB (1 l.) and H_2O (1 l.), fraction size being 16 ml. Fractions 31–41 (TOD, A_{260nm} 1900 units) were pooled and concentrated to dryness in vacuo.

UpA. The above residue was dissolved in acetic anhydride (50 ml) and the solution was allowed to stand at 43° for 6 days (within 18 hr, a complete solution resulted). The solution was concentrated to dryness in vacuo and the residue was dissolved in methanol (50 ml) saturated with ammonia at 0°. The solution was allowed to stand at room temperature overnight and concentrated to dryness. The residue was applied to a DEAE cellulose column (15 × 3 cm). Elution was performed first with H_2O (1 l.), followed by 0.1 *M* TEAB solution (700 ml). The fraction containing UpA was pooled (A_{260nm} 1400 units) and concentrated to dryness, yield 92%, based on the assumption that the molecular extinction coefficient of **9** was 15,000. On the Varian LCS 1000 column chromatography this sample behaved similarly to an authentic sample of UpA.[20]

Digestion of the Dinucleoside Monophosphate (UpA) with Pancreatic Ribonuclease. The reaction mixture contained 40 μl of the sample of UpA (A_{260} nm 20 units), 20 μl of RNase (1 mg/1 ml), and 40 μl of Tris·HCl (pH 7.5) in a total volume of 100 μl. This mixture was incubated at 37° for 24 hr. After this period, paper electrophoresis (the conditions were the same as above) showed that UpA was completely hydrolyzed with the enzyme to afford uridine 3'-phosphate and adenosine in a molar ratio of 1:1.

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Registry No.—**1**, 54618-02-9; **2**, 54618-03-0; **3**, 58-96-8; **4**, 54618-04-1; **5**, 54618-05-2; **6**, 54618-06-3; **7**, 54618-07-4; **8**, 54618-08-5; **9**, 54618-09-6; **10**, 54618-10-9; **11**, 54657-21-5; **14**, 54618-11-0; **16**, 58-61-7; **17**, 54657-22-6; **18**, 54618-12-1; **19**, 33485-36-8; **20**, 54618-13-2; **21**, 54618-14-3; **23**, 54618-15-4; **24**, 23197-78-6; **27**, 3256-24-4; trityl chloride, 76-83-5; benzoyl chloride, 98-88-4; 2',3'-*O*-isopropylideneuridine, 362-43-6.

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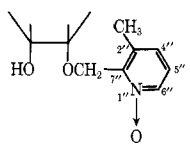

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(19) A column used was packed with PA 38 pellicular anion exchange resin (column size 300 cm × 1 mm); temperature 70°; flow rate 10 ml/hr; elution was performed by a linear gradient from 0.02 *M* KH_2PO_4 (pH 3.25) to 1.0 *M* KH_2PO_4 (pH 3.85); initial gradient chamber volume, 40 ml; gradient delay, 10 min. Under these conditions, the retention time of UpA was 56 min (a single peak).