Protecting Groups. 7. A Novel Type of Neighboring Group Participation Involving Pyridine *N*-Oxides in Acylation and Phosphorylation. 1[1]

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Competitive acylation of 2-(ω-hydroxyalkyl)pyridine 1-oxides **3a** and **3b** and benzyl alcohol (**13**) with *p*-nitrobenzoyl chloride in pyridine afforded 2-[ω-(*p*-nitrobenzoxy)alkyl]pyridine 1-oxides **4a** and **4b** almost exclusively. Even in the presence of a large excess (viz. 30 molar excess) of **13** the same result was obtained. Analogous competitive phosphorylation of 4-methoxy-2-pyridinemethanol 1-oxide (**5**) and 1-butanol (**17**) with 2,2,2-trichloroethyl phosphorodichloridate (**16**) gave rise to a 55.6% yield (based on **16**) of *n*-butyl 4-methoxy-2-picolyl 2,2,2-trichloroethyl phosphate 1-oxide (**7**) via 4-methoxy-2-picolyl 2,2,2-trichloroethyl phosphorochloridate 1-oxide (**6**). A mechanism of the selective acylation, together with a possibility of the application of the 4-methoxy-2-picolyl 1-oxide group as the 2′-O-protecting group which might assist phosphorylation at 3′-OH of the ribonucleoside, is discussed.

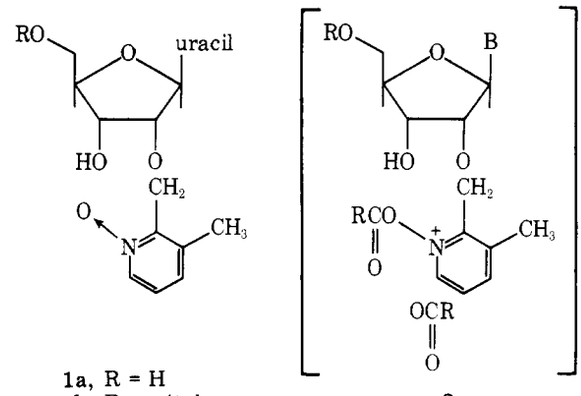
Introduction of a blocking group often causes reduction in the reactivity of the adjacent functional group due, mainly, to steric hindrance by the group introduced.[2] For example, condensation of 2′-*O*-(1-ethoxyethyl)uridine with 2-cyanoethyl 2′,5′-di-*O*-(1-ethoxyethyl)uridine 3′-phosphate afforded only 2′,5′-di-*O*-(1-ethoxyethyl)uridylyl-(3′–5′)-2′-*O*-(1-ethoxyethyl)uridine 2-cyanoethyl ester. The fact that no 3′–3′ isomer was detected in the reaction mixture indicates the inhibition of phosphorylation on the 3′-OH by the 2′ substituent of the nucleoside.[3]

Although this feature permits the use of nucleosides unprotected at 3′-OH in the synthesis of diribonucleoside monophosphates by the "two-step procedure",[4,5] this often offers serious problems in oligoribonucleotide synthesis. Thus, DCC or TPS-activated phosphorylation of 2′,5′-di-*O*-methoxytetrahydropyranyladenylyl-(3′–5′)-2′-*O*-methoxytetrahydropyranyladenosine with 2-cyanoethyl phosphate did not occur. An attempt to condense 2′,3′-*O*-methoxymethyleneuridine 5′-phosphate and 2′,5′-di-*O*-methoxytetrahydropyranyladenylyl-(3′–5′)-2′-*O*-methoxytetrahydropyranyladenosine has failed.[6] These results can be attributed to the steric hindrance of the bulky ketal function adjacent to the free 3′-OH group. Thus, further progress may not easily be possible without introduction of a better 2′-O protecting group. In order to solve this problem we have concentrated our efforts in search of a novel type of 2′-*O* protection that is capable of assisting 3′-*O* phosphorylation.

It is known that the oxygen atom of pyridine 1-oxide is sufficiently nucleophilic to react rapidly with acyl chloride (at room temperature in $CDCl_3$) to form *N*-acyloxypyridinium chloride which is recognized as a good acylating agent.[1b,7] Therefore, pyridine 1-oxide may be a possible candidate as a 2′–O protecting group which would be acylated to form the *N*-acyloxypyridinium salt.[8] The latter would acylate (or phosphorylate) the adjacent 3′-OH group.

In a previous report from our laboratory,[9] acylation or phosphorylation of 2′-*O*-(3-methyl-2-picolyl 1-oxide)uridine (**1a**, R = H) was described. Although we did not discuss the mechanism, the acylation on the 3′-OH group might have proceeded via the *N*-acyloxypyridinium intermediate **2**. It is, however, difficult to assess exactly the possible neighboring participation of the *N*-oxide on acylation because of the complexity of the system.[10]

In order to show definitely the participation in acylation and the high nucleophilicity of the *N*-oxide group toward sp^2 carbon or sp^3 phosphorus, competitive acylation was designed using a model system. Finally, competitive acylation between 3-methyl-2-picolyl 1-oxide protected pyrimidine nucleosides and benzyl alcohol (**13**) with benzoyl halide was attempted.



1a, R = H
b, R = trityl

2

Results

A mixture of benzyl alcohol (**13**) and 2-(ω-hydroxyalkyl)-pyridine 1-oxides **3a** and **3b** was treated with *p*-nitrobenzoyl chloride (**14**) in pyridine. We chose **3a, 3b**, and **13** as substrates for the competitive acylation because of their apparent steric similarity. *p*-Nitrobenzoyl chloride (**14**) was selected as the acylating agent because of its strong ultraviolet-absorbing nature and different mobilities on thin-layer chromatography of the expected products, benzyl *p*-nitrobenzoate (**8**) and 2-[ω-(*p*-nitrobenzoxy)alkyl]pyridine 1-oxides **4a** and **4b** (Scheme I).

A solution of 1 mmol (1 equiv) of 2-(ω-hydroxyalkyl)pyridine 1-oxides **3a** or **3b** and a 30 molar excess of **13** in chloroform was treated with ~0.55 equiv of **14** in the presence of a limited amount of pyridine overnight at room temperature. The reaction mixture was analyzed by thin-layer chromatography using *p*-nitrotoluene (**10**) as the internal standard. The TLC plate was developed first by benzene and then by $CHCl_3$–EtOH to obtain a clear separation of each component (including the internal standard) of the mixture. The relative ratio of a pair of products (one partner being the internal standard **10**) was calculated on the basis of $A_{262\ nm}$ values exhibited by the extract of each spot. The results are listed in Tables I and II which show that, under the above conditions, **4a** or **4b** was obtained almost exclusively. Even in the presence of a 30 molar excess of **13** acylation nearly exclusively occurred on the ω-hydroxyl group. The preferential acylation of the ω-hydroxyl group of the side chain was thus observed.

Analogous treatment of 2-pyridinemethanol 1-oxide (**3a**) with 2,2,2-trichloroethyl phosphorodichloridate (**16**) in the presence of 1-butanol (**17**) afforded *n*-butyl 2-picolyl 2,2,2-trichloroethyl phosphate 1-oxide (**18**). In this case, however,

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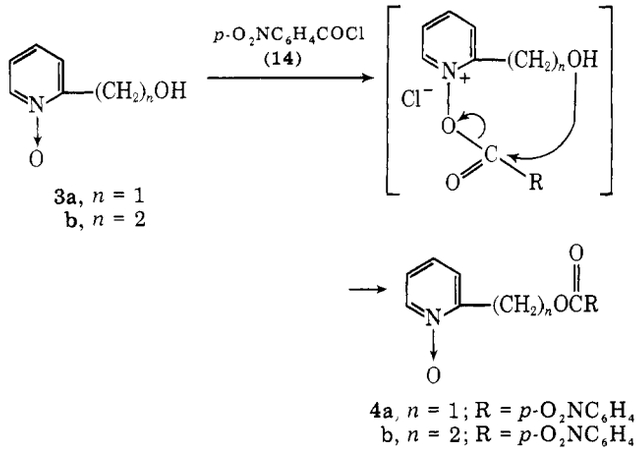
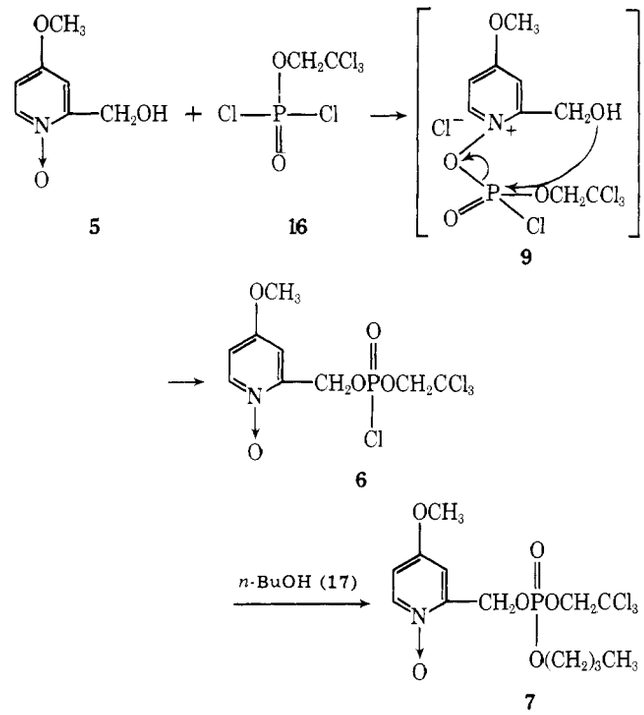
Scheme I



Scheme II



the yield never exceeded 11.9%. On the other hand, when 4-methoxy-2-pyridinemethanol 1-oxide (**5**) and **17** were used as substrate, competitive reaction under comparable conditons afforded *n*-butyl 4-methoxy-2-picolyl 2,2,2-trichloroethyl phosphate 1-oxide (**7**) in 56% (isolated) yield. In the absence of **17** this reaction afforded 4-methoxy-2-picolyl 2,2,2-trichloroethyl phosphorochloridate 1-oxide which was capable of phosphorylating **17** rapidly to give **7**, whereas, under comparable conditions, 1-butanol did not react with **16** to any considerable extent. Thus, again the preferential phosphorylation of the ω-hydroxyl group of **5** (but not **3a**) with **16** was observed (Scheme II).

Finally, a similar competive acylation of 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (**1b**) and **13** with *p*-nitrobenzoyl chloride (**14**) in pyridine and chloroform at room temperature was found to give, contrary to our expectation, benzyl *p*-nitrobenzoate (**8**) as the exclusive product, **1b** being recovered intact in quantitative yield. In sharp contrast, acylation of 3′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityl-N^4-benzoylcytidine (**11**, 1 equiv) and **13** (2 equiv) with benzoyl bromide (**15**, 1 equiv) in the presence of 2,4,6-trimethylpyridine afforded a 19% yield (based on **15**) of 3′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityl-$O^{2'}$,N^4-dibenzoylcytidine (**12**), whose anomeric proton signal appeared relatively downfield, as compared to that of **11**. The structure of **12** was also confirmed by elemental analysis (Scheme III).

Table I. Amounts of Benzyl *p*-Nitrobenzoate (8) and 2-Picolyl 1-Oxide *p*-Nitrobenzoate (4a) Formed in Competitive Acylation with *p*-Nitrobenzoyl Chloride (14) between 2-Pyridinemethanol 1-Oxide (3a) and Benzyl Alcohol (13)[a]

Run	1	2	3	4	5
3a, mmol	1.00	1.02	1.02	1.02	1.03
13, mmol	5.13	10.18	15.1	20.1	30.1
14, mmol	0.55	0.54	0.56	0.57	0.54
A_{4a}/A_{10}[b]	3.31	3.05	3.71	3.16	3.39
A_8/A_{10}[b]	0.18	0.14	0.13	0.10	0.09
Yield of **4a**, mg	112	98	125	106	115
Yield of **4a**, %[c]	75.1	67	81	69	77
Yield of **8**, mg	9.0	7.0	6.5	5.0	4.5
Yield of **8**, %[c]	6.4	5.0	4.5	3.5	3.1

[a] In the presence of a limited amount of pyridine (0.56–0.77 mmol) in chloroform (4 mL) at 23 °C. [b] For the definition of A_{4a}/A_{10} and A_8/A_{10}, see the Experimental Section. [c] Based on the acylating agent **14**. Besides **4a** (67–81%) and **8** (4.5–9.0%), **14** was recovered as *p*-nitrobenzoic acid in 15–20% yields.

Table II. Amounts of Benzyl *p*-Nitrobenzoate (8) and 1-(2-Pyridyl 1-oxide)-2-(*p*-nitrobenzoxy)ethane (4b) Formed in Competitive Acylation with *p*-Nitrobenzoyl Chloride (14) between β-(2-Pyridyl 1-oxide)ethanol (3b) and Benzyl Alcohol (13)[a]

Run	1	2	3	4	5
3b, mmol	0.968	1.03	1.32	1.06	1.05
13, mmol	0.986	3.22	5.42	15.1	30.4
14, mmol	0.542	0.538	0.560	0.548	0.553
A_{4b}/A_{10}[b]	3.66[c]	3.39[c]	3.09[d]	3.07[d]	3.10[d]
A_8/A_{10}[b]	0.00	*d*	0.00	*d*	0.10
Yield of **4b**, mg	135	117	114	111	112
Yield of **4b**, %[e]	85.2	74.3	71.0	69.3	69.2

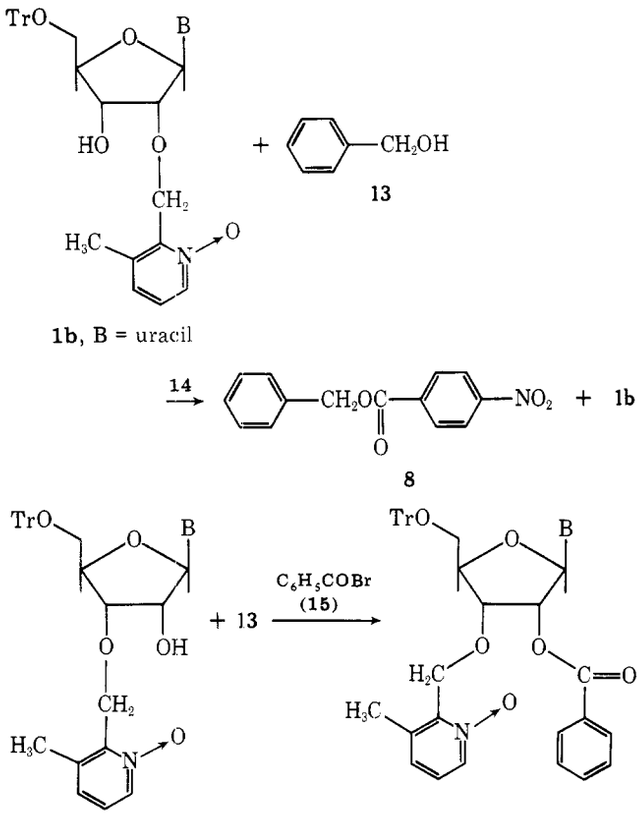
[a] In the presence of a limited amount of pyridine (0.46–0.82 mmol) at 23 °C. [b] For the definition of A_{4b}/A_{10} and A_8/A_{10}, see the Experimental Section. [c] Averaged values of three determinations. [d] Averaged values of two determinations. [e] Based on the acylating agent **14**. Besides **4b** (66–80%), **14** was recovered in 15–25% yields as benzoic acid.

Discussion

It is well established that, under comparable conditions, nucleophilic substitution at an unsaturated center such as ester formation from acyl halide and alcohol is controlled by steric factors associated with both reagents and the nucleophilicity of attacking groups (alcohols). The structure of 2-pyridinemethanol 1-oxide (**3a**) resembles that of benzyl alcohol (**13**) except that the former has an *N*-oxide group. It is reasonable, therefore, to assume that the steric factors associated with both alcohols are nearly equal, and, consequently, the relative rate of the ester formation between two competing alcohols is apparently a reflection of the difference in nucleophilicity of the two.

Ample examples are known that hydrogen-bonded hydroxyl groups behave as stronger nucleophiles than non-hydrogen-bonded hydroxyl groups toward a hard[11] carbonyl center.[12] The existence of weak hydrogen bonding in the alcohol [**13**, **3a**, **3b**, or 4-methoxy-2-pyridinemethanol 1-oxide (**5**)] was indicated by NMR spectra [signals due to respective hydroxyl groups of **13**, **3a** **3b**, and **5** appear at δ ($CDCl_3$) 3.88, 7.43, 5.52, and 5.42]. However, the difference of the degrees in hydrogen bonding is too slight to expect the dramatic difference in the

Scheme III



relative rate of acylation among these alcohols.[13] The only remaining possibility to invoke the explanation of the observed selective acylation of the ω-hydroxyl group of 2-pyridyl 1-oxide alcohols, **3a** and **3b**, to **13** is the neighboring group participation of the *N*-oxide group, assuming that the nucleophilicity of this group toward the sp^2 carbon is higher than that of the hydroxyl group of **13**. This assumption may not be farfetched, because the *N*-oxide group is hard.[14]

As far as we are aware,[15] the present paper is the first to demonstrate that the *N*-oxide group of pyridine 1-oxide may be more nucleophilic (harder) than the hydroxyl group of an alcohol.[16] It is also of interest to note that the nucleophilicity of the *N*-oxide group of **3a** toward the sp^3 center of the phosphorodichloridate may not be high enough to form the corresponding pyridinium intermediate (at room temperature), whereas the *N*-oxide group of **5** was reactive enough to give a pyridinium intermediate (see Scheme II). This trend is in reasonably good agreement with that previously observed.[1b]

In the absence of **13**, 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (**1b**) was acylated to afford the corresponding 3′-*O*-(*p*-nitrobenzoyl) derivative. In its presence, however, benzyl *p*-nitrobenzoate (**8**) was formed under the "standard conditions" (see the Experimental Section), **1b** remaining completely intact.

In order to find the factors causing the remarkable difference in reactivity between **1b** and **3a**, an inspection of the space-filling molecular model was made. The molecular model for the former showed that the 2′-*O*-(3-methyl-2-picolyl 1-oxide) group and the uracil moiety of the former are capable of readily stacking together, the *N*-oxide oxygen sticking out away from the 3′-OH group, and a considerably high barrier has to be overcome to twist the 2′-O protecting group to form a cyclic intermediate (structure **2** in Scheme I) that could transfer the acyl group to the 3′-OH group. Evidence for the strong stacking interaction of the two aromatic rings in **1b** (not in its 3′-O isomer) has already been obtained[9] by the larger hypsochromicity in its UV spectrum and its lower solubility in aqueous methanol.

In regard to the hypsochromicity and the solubility, a similar trend could be observed between 2′-*O*-(3-methyl-2-picolyl)-5′-*O*-trityl-N^4-benzoylcytidine and its 3′-O isomer **11**. The competitive acylation with benzoyl bromide (**15**, 0.9 equiv) between the scarcely stacked[17] nucleoside **11** (1 equiv) and **13** (2.15 equiv) afforded an 18% yield of the corresponding nucleoside **12**, as expected. This fact presumably corroborates the above explanation.

It appears to be an attractive challenge to elaborate a 2′-O protecting group for the ribonucleosides that might be capable of assisting phosphorylation of the 3′-OH group. The present investigation suggests that, to achieve this, at least two requirements must be fulfilled by the protecting group. This should be a heterocyclic *N*-oxide that would be stacked with the aromatic ring of the nucleoside to the least extent when attached to the 2′-oxygen, and the sp^3 phosphorus nucleophilic activity of the oxygen atom of the *N*-oxide must be comparable to or superior to that of 4-methoxy-2-picoline 1-oxide.

Experimental Section

Melting points were determined in capillaries, heated in an oil bath on a Yamato instrument, and are not corrected. Nuclear magnetic resonance (NMR) spectra were determined in chloroform-*d* at 60 MHz on a Hitachi spectrometer, Model R24. Chemical shifts were reported in parts per million downfield from Me_4Si. Mass spectra were obtained on a Hitachi RMU-6E spectrometer. The progress of the reactions was routinely followed either by thin-layer chromatography (TLC) or NMR. TLC was run on glass plates coated with silicic acid in the following systems: solvent A, benzene; solvent B, $CHCl_3$–EtOH (7:1) and the mixture of other proportions was also used. In order to obtain quantitative data, the plate was carefully heated at ca. 150 °C for 3 h prior to use.

2-Pyridinemethanol 1-oxide (**3a**, mp 138–141 °C),[18] β-(2-pyridyl 1-oxide)ethanol (**3b**, mp 93–95 °C, from dry ethyl acetate),[19] γ-(2-pyridyl 1-oxide)propanol [**3c**, mp 52–54 °C, bp 120 °C (0.1 mm)],[20] 2,2,2-trichloroethyl phosphorodichloridate [**16**, 116–118 °C (14 mm)],[21] and benzyl *p*-nitrobenzoate (**8**, mp 84.5–85 °C)[22] were prepared as reported.

4-Methoxy-2-pyridinemethanol 1-Oxide (5). To a solution of 4-methoxy-2-picoline 1-oxide (11 g, 0.071 mol) in chloroform (20 mL) was added acetic anhydride (30 mL). The solution was heated for 4 h at refluxing temperature. The cooled solution was poured into ice and water. The solution was adjusted with saturated Na_2CO_3 to pH 9. The product was extracted with chloroform (40 mL × 3). The dried ($MgSO_4$) solution was concentrated to dryness. The residue was applied to a silica gel (200 g) column. The column was washed with $CHCl_3$–EtOH (1000:15) and the fractions containing 4-methoxy-2-pyridylacetoxymethane were collected. Evaporation of the eluate afforded the desired product. The structure was confirmed by NMR [δ 2.13 (s, 3 H, CH_3CO), 3.83 (s, 3 H, CH_3O), 4.77 (s, 2 H, $-CH_2OAc$), 6.71 (q, 1 H, H_5), 6.88 (q, 1 H, H_3), 8.37 (d, J = 7 Hz, 1 H, H_6)]. The yield was 8.5 g (65%). The above product (8.2 g, 45.3 mmol) was treated with hydrogen peroxide (30%, 7.67 g) in acetic acid (20 mL) at 40 °C for 12 h. After ascertaining by TLC ($CHCl_3$–EtOH, 40:5) that the reaction was complete, the mixture was concentrated to dryness. The residue was treated with saturated methanolic ammonia (40 mL) for 3 h at room temperature. The product was purified on a silica gel column [silica, 50 g; EtOH–$CHCl_3$ (100:1)]. The crude yield was 4.6 g. Crystallization from EtOH–*n*-pentane afforded an analytical sample: mp 135–137 °C; yield 3.0 g (42%); NMR δ 3.83 (s, 3 H, CH_3O), 4.77 (s, 2 H, CH_2OH), 6.63 (m, 1 H), 7.06 (m, 1 H), 8.07 (d, J = 7 Hz, 1 H, H_6). Anal. Calcd for $C_7H_9NO_3$: C, 54.10; H, 5.81; N, 9.03. Found: C, 54.00; H, 5.82; N, 9.05.

1-(2-Pyridyl 1-oxide)-2-(*p*-nitrobenzoxy)ethane (4b). An authentic sample of **4b** was prepared by a standard procedure from *p*-nitrobenzoyl chloride and **3b**: mp 124–126 °C (crystallized from ethanol). Anal. Calcd for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.16; N, 9.72. Found: C, 58.31; H, 4.17; N, 9.72.

4-Methoxy-2-picolyl 2,2,2-Trichloroethyl Phosphorochloridate 1-Oxide (6), HCl Salt. To a solution of 4-methoxy-2-pyri-

Table III. Calibration of Analysis of 8

Run	1	2	3
8, mg	17.0	52.0	147.3
10, mg	53.0	56.3	63.9
Wt_8/wt_{10}[a]	0.309	0.923	2.305
A_8/A_{10}	0.316	0.905	2.156

[a] Wt_8/wt_{10} refers to the ratio of the weight of 8 to that of 10. [b] For the definition of A_8/A_{10}, see the Experimental Section.

dinemethanol 1-oxide (**5**, 155.4 mg, 1 mmol) in chloroform-*d* (1 mL) containing pyridine (90.9 mg, 1.15 mmol) was added a solution of 2,2,2-trichloroethyl phosphordichloridate (**16**, 267.1 mg, 1 mmol) in chloroform-*d* (0.5 mL) with stirring at room temperature. After a 20-min period of stirring, a white precipitate formed which in turn was added with chloroform-*d* (1 mL). The product was collected by filtration. The yield of **6** (HCl salt) was 250 mg (50%). The structure was confirmed after neutralization by NMR (see Table IV).

Competitive Reaction I. Reaction of 2-(ω-Hydroxyalkyl)-pyridine 1-Oxides 3a–c and Benzyl Alcohol (13) with *p*-Nitrobenzoyl Chloride (14). a. 2-Pyridinemethanol 1-Oxide (3a). The following operations were all performed at room temperature (~23 °C). To a solution of 2-pyridinementhanol 1-oxide (**3a**, 125 mg, 1.0 mmol), **13** (554.8 mg, 5.09 mmol), and pyridine (58.8 mg, 0.74) in chloroform (4 mL) was added with stirring a solid sample of **14** (102.8 mg, 0.554 mmol). After a 5-min period of stirring, complete solution resulted. Stirring was continued overnight. The reaction mixture was added to 53.3 mg (0.784 mmol) of *p*-nitrotoluene (**10**) as an internal standard for the subsequent spectrometric analysis. An aliquot (~100 $A_{262\ nm}$ units) was applied to a TLC plate. The same plate was spotted alongside, at separate points (from each other as well as the spot of the reaction mixture), with three respective solutions of the following authentic samples: **10**, **8**,[23] and 2-picolyl 1-oxide *p*-nitrobenzoate (**4a**).[24] These samples were developed first with benzene to a distance of ~15 cm and subsequently (after being redried) developed with solvent B. Spots were visualized under a UV lamp. Each compound including pyridine hydrochloride could be cleanly separated, the order of larger mobility being **10**, **8**, **4a**, and pyridine hydrochloride. Each spot along with a blank spot of the same area was scratched out and transferred to a centrifuge tube. There was then added 4 mL of EtOH. After being well shaken for 25 min, silica was centrifuged off (2600 rpm, 30 min). A 0.3-mL volume of the supernatant layer was pipetted and added to 4 mL of EtOH, and the solution was well shaken. The absorbance of each solution was determined in a quartz cell of 1-cm width at 262 nm, using a solution similarly prepared from the blank spot as a control. The entire procedure (including the competitive acylation) was repeated five times. In one run, the following $A_{262\ nm}$ values were obtained (A_{10} is short for the $A_{262\ nm}$ value of **10** and so forth): A_8, 0.045; A_{4a}, 0.584, and A_{10}, 0.181. Therefore, A_8/A_{10} (a ratio of A_8 to A_{10}) = 0.257; A_{4a}/A_{10} = 3.32.[25] The averaged value of five runs is listed in Table I.

Similar experiments were performed by the use of 1.099 g (9.97-fold, experiment 2), 1.6387 g (14.82-fold, experiment 3), 2.179 g (19.83-fold, experiment 4), or 3.259 g (29.30-fold, experiment 5) of **13**, other conditions being virtually the same as the above. Each experiment was duplicated. A_{4a}/A_{10} and A_8/A_{10} were similarly obtained and also listed in Table I.

b. (2-Pyridyl 1-oxide)ethanol (3b). Parallel competitive acylation was performed with **3b**. Each experiment was carried out three times. The averaged values are listed in Table II.

c. γ-(2-Pyridyl 1-oxide)propanol (3c). Parallel (but preliminary) competitive acylation was performed with **3c**. A similar trend as in the case of a and b above was observed.

d. Calibration of the Above Analysis. In order to calibrate the above analysis, an artificial solution prepared by dissolving various amounts of 8 together with a definite amount of *p*-nitrotoluene (**10**) in 3 mL of chloroform was analyzed. Each A_8/A_{10} value obtained is given in Table III. When these are plotted against wt_8/wt_{10} (ratio of the weight of 8 to that of **10**) as abscissa, they are located in a straight line which coincides with the origin (namely, a calibration curve for 8 was obtained but is not shown). A good calibration curve for **4a** or **4b** was analogously obtained.

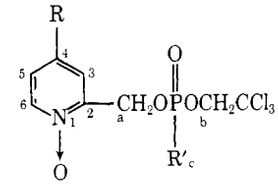
II. Reaction of 4-Methoxy-2-pyridinemethanol 1-Oxide (5) and 1-Butanol (17) with 2,2,2-Trichloroethyl Phosphorodichloridate (16). **16** (140 mg, 0.525 mmol) dissolved in chloroform (1 mL) was added with stirring to a cooled (~15 °C) chloroform solution (5 mL) of **5** (153.1 mg, 0.99 mmol) and 1-butanol (**17**, 85 mg, 1.15 mmol) containing 2,4,6-trimethylpyridine (collidine, 92.8 mg, 0.776 mmol). The mixture was allowed to stand at room temperature for 24 h and then added with vigorous stirring to $NaHCO_3$ solution (pH 10, 20 mL). The chloroform layer was separated and concentrated to dryness. The residue was purified by preparative TLC (silica gel; solvent system $CHCl_3$–EtOH, 7:1). The yield of *n*-butyl 4-methoxy-2-picolyl 2,2,2-trichloroethyl phosphate 1-oxide (**7**) was 124 mg (55.6% yield, based on the phosphorylating agent **16**). The structure of **7** was confirmed by NMR (see Table IV) and mass spectrometry: *m/e* 405, 407 (M − O); 370, 372 (M − OCl).

III. Reaction of 2-Pyridinemethanol 1-Oxide (3a) and 1-Butanol (17) with 2,2,2-Trichloroethyl Phosphorodichloridate (16). To a magnetically stirred solution of **3a** (127.1 mg, 1.02 mmol), 1-butanol (**17**, 92.8 mg, 1.25 mmol), and collidine (128.4 mg, 1.06 mmol) in $CHCl_3$ (5 mL) was added, with slight cooling (overcooling may result in no reaction), a $CHCl_3$ solution (1 mL) of **16** (127.1 mg, 0.48 mmol). The basified (to pH 10 with aqueous solution of $NaHCO_3$) solution was applied to two TLC plates (silica gel; solvent system $CHCl_3$–EtOH, 10:1). The band corresponding to *n*-butyl 2-picolyl 2,2,2-trichloroethyl phosphate 1-oxide (**18**) (whose structure was confirmed by NMR, see Table IV) was scratched out and extracted with $CHCl_3$ (4 mL). Silica was filtered off. Concentration of the filtrate left an almost pure sample of **18** (the purity was checked by NMR): mass spectrum *m/e* 392.391 (M), 375.377 (M − O), 306.308 (M − Cl), 340.342 (M − OCl); yield 22.4 mg (11.9% on the basis of **16**).

IV. Reaction of 2′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-trityluridine (1b) and 13 with *p*-Nitrobenzoyl Chloride (14). Reaction of **1b**[9] (318.9 mg, 0.525 mmol) and **13** (121.4 mg, 1.124 mmol) with **14** (82.2 mg, 0.759 mmol) in pyridine (42.3 mg, 0.523 mmol) and chloroform (4 mL) at room temperature (overnight) gave, after separation on TLC (solvent B), **8** (mp 83.5–84.5 °C)[23] as the exclusive product, **1b** being recovered intact in quatitative yield. The reaction mixture was also analyzed according to the above assay method using 56 mg of **10** as the internal standard. The value (the averaged value of three runs) of A_8/A_{10} was obtained, this value corresponding to 48 mg (by the use of the calibration curve) of 8 (42.1%, on the basis of **14**).

3′-*O*-(3-Methyl-2-picolyl 1-oxide)-5′-*O*-trityl-*N*⁴-benzoylcytidine (11). To a solution of N^4-benzoylcytidine[26] (24.5 g, 70.6

Table IV. Proton Chemical Shifts for 2-Picolyl 2,2,2-Trichloroethyl Phosphate 1-Oxides[a]



Compd	Chemical shifts (ppm) and coupling constants (Hz)						
	H-6	H-3	H-5	H_a	H_b	H_c[b]	OMe
6, R = OMe; R′ = Cl	8.12 d (7.2)[c]	7.06 d (3.2)	6.85 q (7.2, 3.2)	5.42 d (7.6)	4.74 d (7.2)		
7, R = OMe; R′ = *n*-BuO	8.24 d (7.4)	7.14 d (3.4)	6.90 q (7.2, 3.4)	5.43 d (7.7)	4.67 d (7.0)	4.25 q	3.91 s
18, R + H; R′ = *n*-BuO	8.3			5.41 d	4.65 d	4.24 q	

[a] In $CDCl_3$ at room temperature. [b] H_c, $-OCH_2$ in R′ = OC_4H_9. [c] Number in parentheses refers to the coupling constant.

mmol) in DMF (400 mL) was added, with stirring, successively stannous chloride dihydrate (400 mg) and 1-oxido-2-pyridyldiazomethane, prepared from 40 g (131.1 mmol) of the *p*-tosylhydrazone of 3-methyl-2-formylpyridine 1-oxide.[24] The stannous chloride was added twice (400 mg, each) at the interval of a 2-h period. The stirring was continued at room temperature overnight. The reaction mixture was concentrated to leave a residue which was dissolved in chloroform (200 mL) and applied to a column of silica gel (200 g). The column was washed with 1.5 L of chloroform (the eluate being discarded) and then washed with 1.5 L of $CHCl_3$–EtOH (10:1). Concentration of the latter fraction gave a mixture of 2′-*O*- and 3′-*O*-(3-methyl-2-picolyl 1-oxide) nucleosides. Attempted fractional crystallization failed from Me_2SO, but a crystalline isomeric mixture having correct combustion values was obtained. The yield was 29 g (88%). Anal. Calcd for $C_{23}H_{24}N_4O_9 \cdot H_2O$: C, 56.79; H, 4.94; N, 11.52. Found: C, 56.88; H, 5.26; N, 11.33.

A suspension of the isomeric mixture (2 g, 57.6 mmol) in pyridine (400 mL) was treated with triphenylchloromethane (27 g, 96 mmol) at 40 °C for 3 days. The mixture was then concentrated to leave a residue which was dissolved in 200 mL of satruated Na_2CO_3 solution. The solution was treated with chloroform (3 × 300 mL). The dried (Na_2SO_4) chloroform solution was concentrated to dryness. The residue was applied to a column of silica gel (700 g) which was washed with $CHCl_3$–EtOH (100:3). From the fast traveling fraction, the 2′-O isomer was obtained as a homogeneous (in solvent B) foam (8.9 g, 21%): NMR (Me_2SO-d_6) δ 2.42 (s, 3 H, 3″-CH_3[27]), 6.02 (s, 1 H, $H_{1'}$). Anal. Calcd for $C_{42}H_{38}N_4O_7 \cdot 0.5H_2O$: C, 70.09; H, 5.28; N, 7.78. Found: C, 70.15; H, 7.73; N, 7.69.

From the slower traveling fraction, **11** was obtained as a homogeneous (in solvent B) foam (25 g, 61%): NMR (Me_2SO-d_6) δ 2.34 (s, 3 H, 3″-CH_3[27]), 5.87 (s, 1 H, $H_{1'}$). According to Reese's rule,[28] an isomer whose anomeric proton signal appears comparatively upfield (5.87 vs. 6.02 ppm) has been assigned as 3′-O isomer. Anal. Calcd for $C_{42}H_{38}N_4O_7 \cdot 0.5H_2O$: C, 70.09; H, 5.28; N, 7.78. Found: C, 70.11; H, 5.52; N, 7.53.

V. Competitive Reaction of 11 and 13 with Benzoyl Bromide (15). **15** (172.8 mg, 0.944 mmol) dissolved in chloroform (4 mL) was added with stirring to a cooled (10 °C) chloroform solution (8 mL) of **11**[26] (724 mg, 1.02 mmol) and **13** (232 mg, 2.14 mmol) containing 137.7 mg (1.13 mmol) of collidine. The mixture was kept at room temperature overnight. The mixture was added with $NaHCO_3$ (500 mg). TLC examination showed that the reaction mixture contained 3′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityl-$O^{2'}$,N^4-dibenzoylcytidine (**12**) and benzyl benzoate in addition to the starting material. The mixture was applied to a silica gel column (silica, 20 g; solvent system $CHCl_3$–EtOH, 100:3). The fraction containing the nucleoside **12** was collected and the solvent was stripped off to leave **12** (145.4 mg, 19%, based on **15**). Crystallization from *n*-C_6H_{14}–$CHCl_3$ gave an analytical sample of **12**: NMR ($CDCl_3$) δ 2.09 (s, CH_3), 5.44 (d, J = 2.4 Hz). Anal. Calcd for $C_{49}H_{42}N_4O_8 \cdot 2H_2O$: C, 69.33; H, 5.42; N, 6.60. Found: C, 69.10; H, 5.13; N, 6.40.

Registry No.—**1b**, 54618-09-6; **3a**, 10242-36-1; **3b**, 64364-85-8; **3c**, 64364-84-7; **4a**, 50808-24-2; **4b**, 64364-96-1; **5**, 64364-95-0; **6**, 64364-94-9; **6**·HCl, 64364-93-8; **7**, 64364-92-7; **8**, 14786-27-7; **11**, 64364-91-6; **12**, 64364-90-5; **13**, 100-51-6; **14**, 122-04-3; **15**, 618-32-6; **16**, 18868-46-7; **17**, 71-36-3; **18**, 64364-89-2; 4-methoxy-2-picoline 1-oxide, 6890-60-4; acetic anhydride, 108-24-7; 4-methoxy-2-pyridylacetoxymethane, 16665-37-5; N^4-benzoylcytidine, 13089-48-0; 1-oxido-2-pyridyldiazomethane, 50908-23-1; 2′-*O*-(3-methyl-2-picolyl 1-oxide)nucleoside, 64364-87-0; 3′-*O*-(3-methyl-2-picolyl 1-oxide)nucleoside, 64364-86-9; 2′-*O*-(3-methyl-2-picolyl 1-oxide)-5′-*O*-trityl-N^4-benzoylcytidine, 64364-88-1.

References and Notes

(1) (a) Presented in part at the 3rd National Meeting of Nucleic Acid Chemistry in Sapporo, Japan, Sept 1975; (b) for part 6, see Y. Mizuno and T. Endo, *J. Org. Chem.*, accompanying paper in this issue.

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(15) Pertinent to the present discussion is the work by Omura et al. who have observed that the competitive reaction of trifluoroacetic anhydride with methyl sulfoxide and benzhydryl alcohol (at −60 °C) gave rise almost exclusively to di(trifluoromethyl)sulfonium trifluoroacetate. It is safely assumed that N → O and S → O, whose common feature is to bear a dative bond, are better nucleophiles than the OH group of alcohol toward the "hard" carbonyl center [K. Omura, A. K. Sharma, and D. Swern, *J. Org. Chem.*, **41**, 957 (1976)].

(16) Preliminary competitive acylation using methanol or ethanol was performed to give exclusively the esters of **3a–c**. For example, competitive acylation between ethanol (100 mg) and **3b** (300 mg) with benzoylimidazolide hydrochloride also exclusively gave the ester **10** corresponding to **3b**. Gas chromatographic examination (for conditions, see footnote 19 of ref 9) showed the absence of ethyl *p*-nitrobenzoate in the reaction mixture.

(17) By analogy to the case of a pair of **1b** and its 3′-O isomer, as well as by analogy to the observation of Christensen et al. [L. F. Christensen and A. D. Broom, *J. Org. Chem.*, **37**, 3398 (1972)], it is highly probable that **11** may be less stacked relative to the 2′-O isomer.

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(25) The respective ratio (A_8/A_{10} or A_{4a}/A_{10}), calculated on the basis of the amount of *p*-nitrotoluene (**10**) which had been actually added to the reaction mixture (in the case of the above example, 53.3 mg), was standardized to the value per 50 mg of **10**. The latter was further standardized to the value per 100 mg of *p*-nitrobenzoyl chloride. For example, since, in the example, 102.5 mg of the latter was actually used, a value of 0.257 for A_8/A_{10} was obtained by 0.045/0.181 × 53.3/50 × 100/102.5. Other ratios were similarly obtained.

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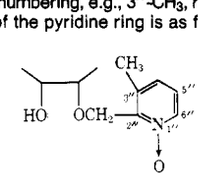
(27) Double prime for the numbering, e.g., 3″-CH_3, refers to the protecting group, and the numbering of the pyridine ring is as follows.



(28) C. B. Reese and D. R. Trenthan, *Tetrahedron Lett.*, 2459 (1965).