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Protecting Groups in Nucleosides and Nucleotides Synthesis. VIII. Synthesis of (4-Substituted 2-Picolyl 1-Oxide)halides and 2′- and 3′-*O*-(4-Substituted-2-picolyl 1-Oxide)nucleosides[1]

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A number of alkylating agents of the 4-substituted pyridine *N*-oxide series, such as (4-nitro-, 4-methylthio-, or 4-methoxy-2-picolyl 1-oxide)halide, have been prepared. Treatment of 2′,3′-*O*-(dibutylstannylene)uridine or 2′,3′-*O*-(dibutylstannylene)adenosine with the alkylating agents afforded the corresponding 2′-*O*- and 3′-*O*-(4-substituted 2-picolyl 1-oxide)nucleosides. 2′-*O*-(4-Nitro-2-picolyl 1-oxide)uridine was converted to the 4-methoxy-2-picolyl derivative on treatment with methanolic sodium methoxide at 60°. However, it was found that the treatment of adenosine with sodium hydride in DMF, followed by alkylation with (4-methoxy-2-picolyl 1-oxide)chloride gave a more satisfactory yield (56.4%) of 2′-*O*-(4-methoxy-2-picolyl 1-oxide)adenosine. 2′-*O*-(4-Methylthio-2-picolyl 1-oxide)adenosine could be similarly prepared in 36.7% yield. 4-Methoxy-2-picolyl protection was removable under milder conditions than 2-picolyl 1-oxide protection. Phosphorylation of **17** with phosphoryl chloride in triethyl phosphate gave the corresponding 5′-phosphate in 57.8% yield.

Keywords——4-substituted 2-picolyl 1-oxide; protecting group in nucleotide synthesis; acetic anhydride rearrangement of α-picolyl 1-oxide; 2′-*O*-substituted adenosine; 2′-*O*-substituted uridine; 4-methoxy-2-pyridyl carbinol 1-oxide; 4-methylthio-2-pyridyl carbinol 1-oxide; 4-nitro-2-pyridyl carbinol 1-oxide

We have reported a procedure for the introduction of a 2-picolyl 1-oxide group at the 2′-hydroxyl group of nucleosides by treatment with (3-methyl-2-pyridyl 1-oxide)diazomethane in the presence of stannous chloride.[3] The diazomethanes were prepared by Bamford's procedure,[4] from the corresponding *p*-tosyl-hydrazones of 2-formylpyridine 1-oxides.[5] It turned out, however, that (4-substituted pyridine-1-oxide)diazomethanes could not be prepared by a similar procedure. Therefore, we investigated two other procedures; treatment of 2′,3′-di-*O*-(dibutylstannylene)nucleosides with alkylating agents which had been developed by Moffatt and co-workers,[6] and alkylation of sodium hydride-treated nucleosides with the alkylating agents.[7] For this purpose, we prepared a number of (4-substituted 2-picolyl 1-oxide)halides (**7**—**11**) from a common starting material, 4-nitro-2-formylpyridine 1-oxide (**1**).[8]

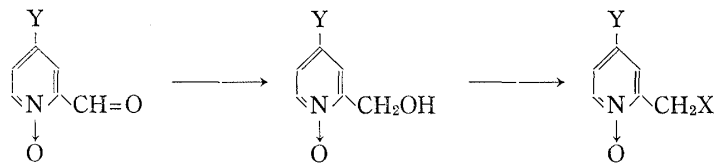


Chart 1

1) For Part VII of this series, see Y. Mizuno and T. Endo, *J. Org. Chem.*, **43**, 684 (1978).
2) Location: *Kita-12 Nishi-6, Kita-ku, Sapporo 060, Japan.*
3) Y. Mizuno, T. Endo, and K. Ikeda, *J. Org. Chem.*, **40**, 1385 (1975).
4) *a*) W.R. Bamford and T.S. Stevens, *J. Chem. Soc.*, **1952**, 4735; *b*) G. Farum, *J. Org. Chem.*, **28**, 870 (1963).
5) Y. Mizuno, T. Endo, and T. Nakamura, *J. Org. Chem.*, **40**, 1391 (1975).
6) D. Wagner, J.P.H. Verheyden, and J.G. Moffatt, *J. Org. Chem.*, **39**, 24 (1974).
7) *a*) E. Ohtsuka, S. Tanaka, and M. Ikehara, *Chem. Pharm. Bull.*, **25**, 949 (1977); *b*) E. Ohtsuka, S. Tanaka, and M. Ikehara, *Synthesis*, **1974**, 453.
8) K.C. Agrawal and A.C. Sartorelli, *J. Med. Chem.*, **21**, 218 (1978).

1: Y=NO_2
2: Y=OCH_3
3: Y=SCH_3
4: Y=NO_2
5: Y=OCH_3
6: Y=SCH_3
7: Y=NO_2, X=Cl
8: Y=NO_2, X=Br
9: Y=NO_2, X=I
10: Y=OCH_3, X=Cl
11: Y=SCH_3, X=Cl

Reduction of 2-formylpyridine 1-oxides (**1**, **2**, or **3**) with sodium borohydride in 80% aq. dioxane gave the corresponding carbinol in excellent yield. Treatment of the carbinol with thionyl chloride gave rise to the chloride (**7**, **10**, or **11**). The bromide or iodide (**8**, or **9**) was prepared by treating the corresponding chloride with sodium halide in DMF at 50° for one hr. These substitution reactions could not be effected in acetone or ethanol even at refluxing temperatures.

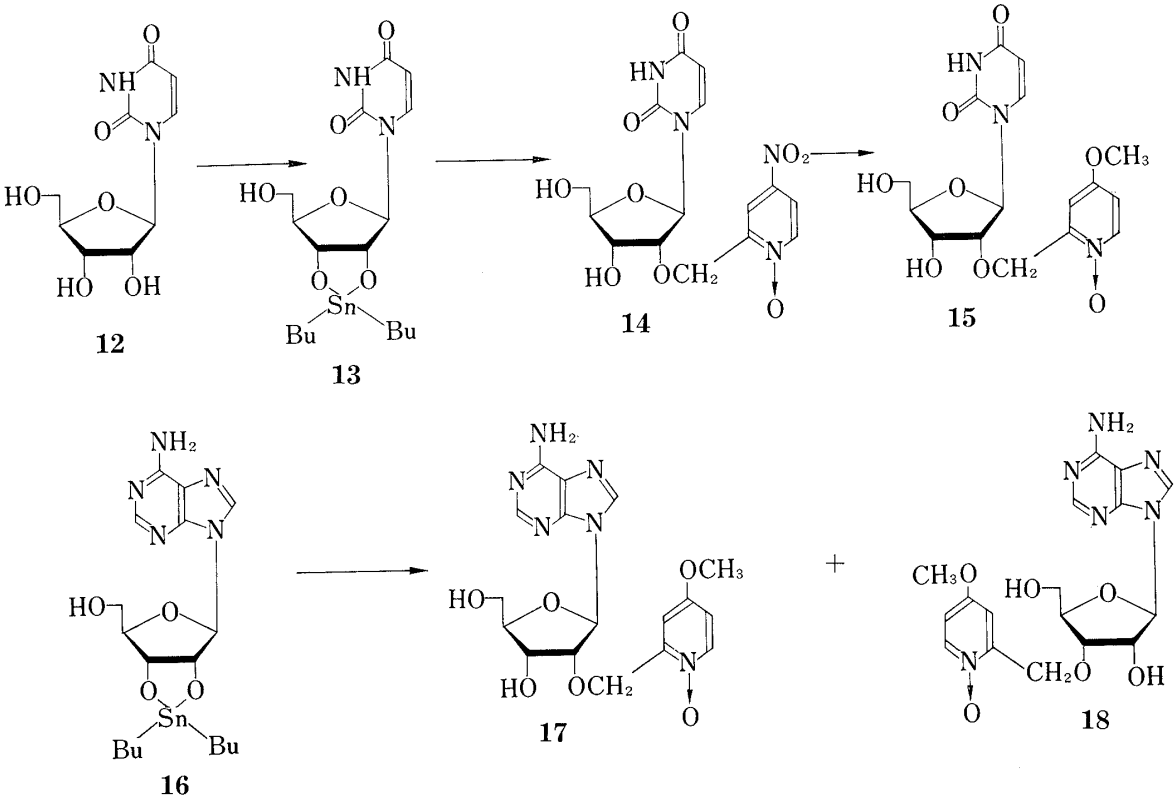


Chart 2

We will next deal with the synthesis of 2′-*O*-(4-substituted-2-picolyl 1-oxide) nucleosides. These compounds were prepared as shown in Chart 2. Thus, 2′,3′-*O*-(dibutylstannylene)-uridine (**13**)[6] was allowed to react with (4-nitro-2-picoyl 1-oxide)chloride (**7**) in DMF at 110° (bath temperature). After work-up, the crude products were purified by column chromatography over silicic acid, eluting with chloroform–ethanol (20: 1). Although we failed to obtain both of the anticipated isomers, one isomer, presumably 2′-*O*-(4-nitro-2-picolyl 1-oxide)uridine (**14**), was obtained as crystals in 12% yield. The structure was confirmed by the spectral [UV[9] and NMR (the signal of the anomeric proton appears at δ 5.98); see Table I] data and combustion values. Treatment of 2′-*O*-(4-nitro-2-picolyl 1-oxide)uridine (**14**) with methanolic sodium methoxide at 60° for 15 min afforded 2′-*O*-(4-methoxy-2-picolyl 1-oxide)uridine (**15**).

9) UV absorption maxima of **15** in MeOH appeared at 330 and 260 nm.

2′-(**17**) and 3′-*O*-(4-Methoxy-2-picolyl 1-oxide) adenosine (**18**) were prepared by two alternative procedures. Treatment of 2′,3′-*O*-dibutylstannylene adenosine (**16**) with (4-methoxy-2-picolyl 1-oxide)chloride (**10**) afforded an isomeric mixture of 2′- and 3′-*O*-substituted adenosine (**17** and **18**), albeit in low yield. Separation of each isomer could be achieved by thick layer chromatography, with methanol as a developing agent. Thus, one isomer was obtained as a homogeneous foam from the faster travelling fraction. The structure was tentatively assigned as the 3′-*O*-isomer (**18**), because in the NMR spectrum, the anomeric proton (δ 5.97) appears comparatively up-field (see below). From the slower travelling band, the 2′-*O*-isomer (**17**), with an anomeric proton signal at δ 6.08, was isolated as a foam. However, the reaction of adenosine, which had been pretreated with one equivalent of sodium hydride with **10** gave a better yield of **17**. This procedure has been developed by Otsuka and *et al.* for the preparation of 2′-*O*-(*o*-nitrobenzyl)nucleosides from adenosine or guanosine.[7] It should be noted that the 3′-*O*-isomer could not be detected in this reaction. Similar alkylation of NaH-treated adenosine with (4-methylthio-2-picolyl 1-oxide)chloride (**11**) gave rise to 2′-*O*-(4-methylthio-2-picolyl 1-oxide)adenosine (**20**) in 36.7% yield.

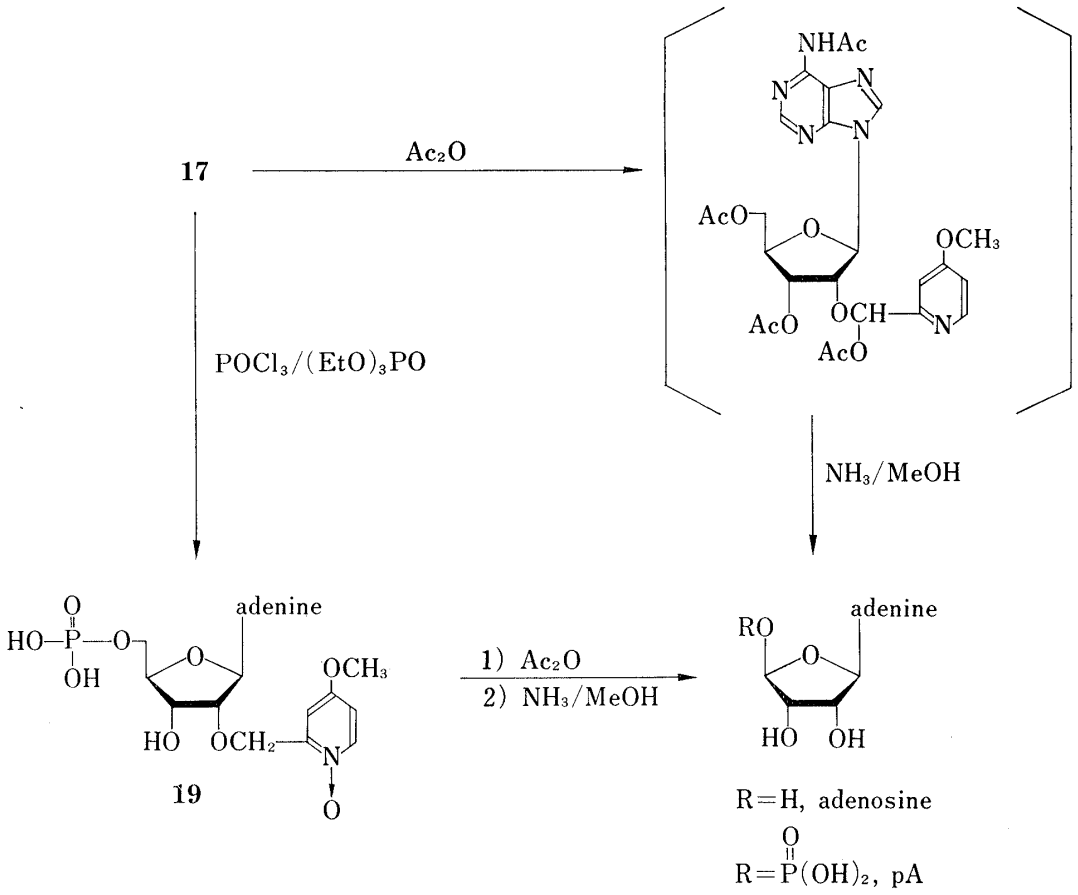
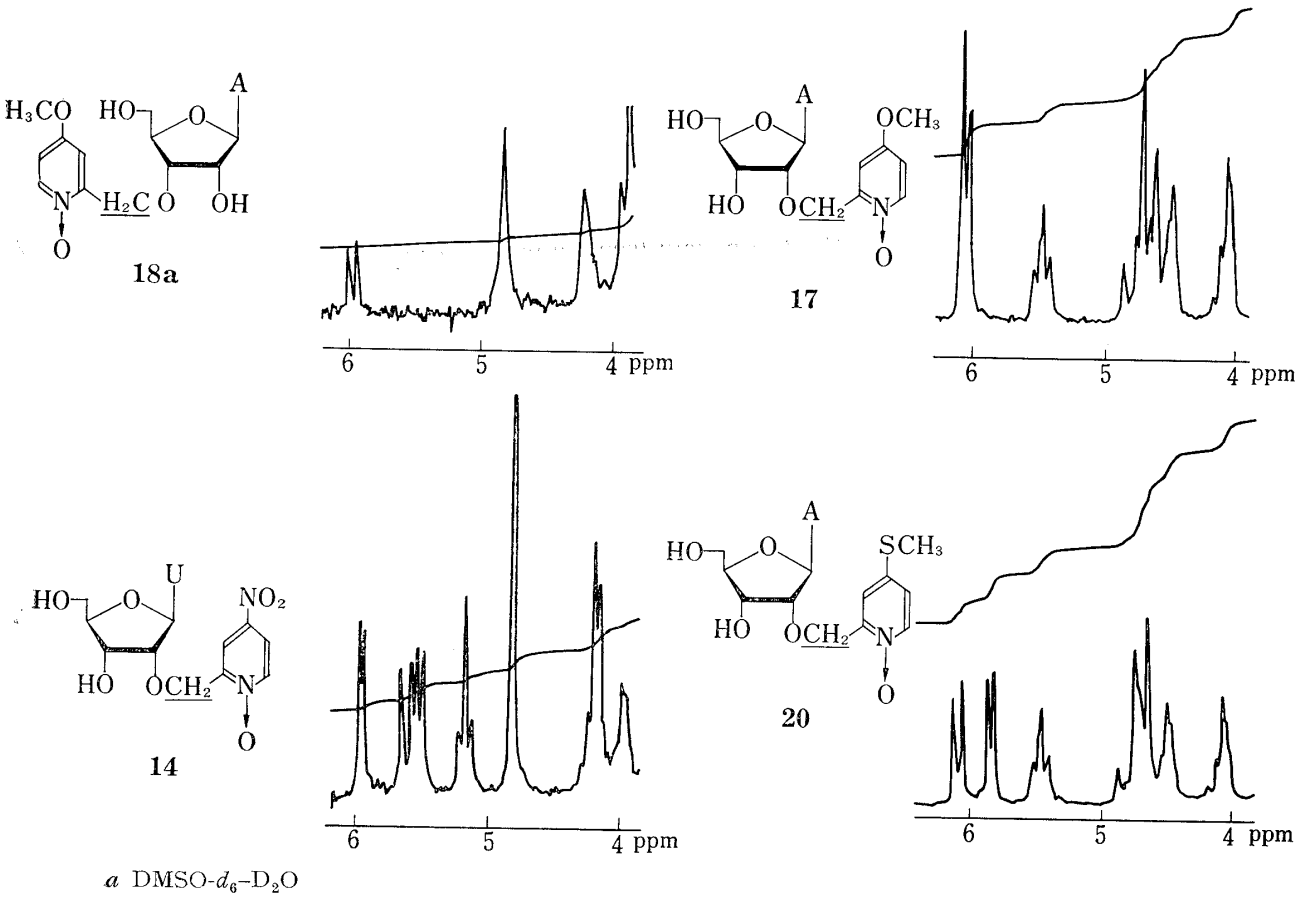


Chart 3

Fig. 1 shows the 4.00—6.00 ppm region of the NMR spectra of 2′-*O*- and 3′-*O*-substituted nucleosides. It is interesting to note that with compounds **17** and **20** and 2′-*O*-benzyladenosine,[10] AB quartets at 4.86 and 5.05 ppm (Jgem., 15 Hz), and at 4.58 and 4.70 ppm (Jgem., 12.5 Hz) indicate the presence of geminal coupling between a pair of protons in benzylic positions, whereas with **14** and **18**, no geminal couplings were observed.

10) L.F. Christensen and A.D. Broom, *J. Org. Chem.*, 37, 3398 (1972).



a DMSO-d_6–D_2O

Fig. 1. The 4-6 ppm Region of NMR various Spectra in DMSO-d_6

Table I. Selected NMR Data DMSO-d_6 (ppm)

Compounds	H_1'	H_2'	H_3'	H_4'	Lit. or Remarks
17	6.08	4.64	4.52	4.07	*a*
18	5.97	4.80	4.21	4.21	
21	6.07	4.63	4.37	4.03	*b*
22	5.94	4.74	4.12	4.12	
23	6.08	4.58	4.42	4.05	*c*
20	6.10	4.65	4.52	4.08	*d*
24	5.93	4.17	4.17	3.89	*e*
25	5.77	4.37	4.00	4.00	
26	5.96	3.97	4.18	3.95	*f*
27	5.80	4.22	3.95	3.95	
14	5.98	4.18	4.18	3.98	*g*

21, 2′-*O*-Benzyladenosine; **22**, its 3′-*O*-isomer; **23**, 2′-*O*-(2-nitrobenzyl) adenosine; **24**, 2′-*O*-(3-methyl-2-picolyl 1-oxide) uridine; **25**, its 3′-*O*-isomer; **26**, 2′-*O*-benzyluridine; **27**, its 3′-*O*-isomer.
a, *f*, the present investigation; *b*, compound (**21**) was prepared by alkylation of NaH-treated adenosine with benzyl-bromide according to ref. 7a while compound (**22**) was prepared by alkylation of adenosine with phenyldiazomethane in the presence of $SnCl_2$–$2H_2O$ according to ref. 10.; *c*, data from ref. 7a; *d*, data from ref. 3; *e*, data from ref. 6.

It is also worthy of note that the signals due to H-3′ and H-4′ in the 2′-*O*-isomers (**17**, **21**, **23**, **20**, **24**, **26**, and **14**) appear at separate positions (see Table I), whereas the corresponding peaks of the 3′-*O*-isomer (**18**, **22**, **25**, and **27**) are overapping and appear as a two-proton multiplet (determined by integration) at around 4 ppm (see Table I and Fig. 1). This is presumably because the benzyl or picolyl 1-oxide group on the 3′-hydroxyl group may induce a considerable downfield shift of the signals due to H-4′, while the peaks due to H-3′ remain virtually unchanged; these blocking groups on 2′-*O*- do not have any effect on the peaks due to H-4′. This characteristic might be useful for the structural assignment of a positional isomer when one of a pair of isomers (2′-*O*- and 3′-*O*-) is not available.

Adenosine could be recovered in excellent yield on treatment of **17** with acetic anhydride at 45° for 13 hr, followed by treatment with methanolic ammonia at room temperature. It was found that 4-methoxy-2-picolyl 1-oxide protection could be removed under milder conditions than 2-picolyl 1-oxide protection.[2)]

Phosphorylation of **17** with phosphoryl chloride in triethyl phosphate according to a reported procedure[11)] afforded the 5′-phosphate of **17** in 57.8% yield, which might be useful for the synthesis of oligonucleotides.

Experimental

General——The melting points were determined on a Yamato melting point apparatus and are uncorrected. The NMR spectra were recorded on a Hitachi NMR spectrometer, model R24. The chemical shifts are given in parts per million downfield from Me_4Si. The mass spectrometer employed was a Hitachi RMU-6E instrument, at an ionizing energy of 80 eV. Elemental analyses were performed by the staff of the Analytical Center of Hokkaido University. Paper chromatography was performed by an ascending technique using solvent system A: iso-C_3H_7OH–$(NH_4)_2SO_4$–H_2O (2: 70: 19). Paper electrophoresis was performed using 0.05 M triethylammonium bicarbonate (pH 7.5) at 30 V/cm. High pressure liquid chromatography was performed on an Varian chromatograph, model LCS 1000, using an AS-Pellionex SAX resin. Abbreviations used in this section are: TEAB, triethylammonium bicarbonate; TLC, thin-layer chromatography; PPC, paper chromatography; PEP, paper electrophoresis; s, singlet; d, bd, broad doublet; m, multiplet; paper chromatography; PEP, paper electrophoresis; TOD, total optical density.

General Procedure for the Preparation of 4-Substituted 2-Pyridylmethanol 1-Oxide (4, 5, and 6)——Solid $NaBH_4$ (388 mg, 10.3 mmol) was added in portions to a solution of 4-substituted 2-formylpyridine 1-oxide (**1**, **2**, or **3**, 22 mmol) with stirring at 0—5°. The reaction was followed by TLC, using $CHCl_3$–EtOH (10: 1) as a solvent system. After ascertaining by TLC that the reaction was complete (it took about 40 min), the reaction mixture was neutralized with 2 N H_2SO_4 solution and filtered. The filtrate was concentrated to dryness. The residue was recrystallized from an appropriate solvent (H_2O for **4**, aqueous acetone for **5**, and EtOH–*n*-pentane for **6**).

Table II. Melting points, Yields, and Combustion Values of Carbinols (**4**, **5**, and **6**)

Compd.	mp, °C	Yield (%)	Calcd %				Found %			
			C	H	N	S	C	H	N	S
4,	157—158	74.6	42.35	3.53	16.47		42.35	3.50	16.53	
5,[b)]	135—137	78	54.10	5.81	9.03		54.47	5.63	9.03	
6,	129—130	82	46.65	5.59	7.77	17.79[a)]	47.04	5.68	7.85	18.08

a) For $C_7H_9O_2NS \cdot 1/2H_2O$.
b) Compound (**5**) has also been prepared by another route[*J. Org. Chem.*, **43**, 684 (1978)].

4-Methoxy-2-pyridylmethanol 1-Oxide (5). Alternate Synthesis A——A solution of 4-nitro-2-formylpyridine ethylene acetal 1-oxide[12)] (10 g, 47 mmol) in absolute methanol (450 ml) was treated at 60° with methanolic sodium methoxide, prepared from 1.1 g of sodium and 150 ml of methanol. The resulting solution

11) M. Yoshikawa, I. Kato, and T. Takenishi, *Tetrahedron Lett.*, **1967**, 5065.
12) Y. Mizuno, T. Endo, Y. Inoue, H. Tampo, and A. Takahashi, M. Iigo, A. Hoshi, and K. Kurerani, *Chem. Pharm. Bull.*, **28**, 1584 (1980).

was kept at the same temperature for 20 min. After ascertaining by UV[13] that the reaction was completed, the reaction mixture was neutralized with acetic acid and then concentrated to dryness. The residue was dissolved in H_2O (200 ml) and the aqueous solution was extracted four times with chloroform (4×350 ml). The combined organic layer was dried over $MgSO_4$. The salt was filtered off. The filtrate was concentrated to dryness, providing 9.85 g of crude 4-methoxy-2-formylpyridine ethylene acetal 1-oxide. The structure was confirmed by NMR. The crude sample was dissolved in 20% hydrochloric acid (260 ml). The solution was heated at 110° (bath temperature) for 30 min. The reaction was monitored by TLC (solvent system, $CHCl_3$–EtOH 10: 1; the spot was visualized with Tollen's reagent). The cooled solution was neutralized with solid $NaHCO_3$. The mixture was concentrated to dryness *in vacuo*. The residue was treated with $CHCl_3$ (120 ml×7). Removal of the solvent left 6.6 g of yellow 4-methoxy-2-formylpyridine 1-oxide.

The crude sample (6.6 g) was dissolved in 80% aqueous dioxane solution (200 ml). The cooled (at 0°) solution was treated with solid $NaBH_4$ (840 mg, 22.2 mmol) in portions, over 60 min with stirring. The reduction was monitored by TLC ($CHCl_3$–EtOH 10: 1). When the reduction was completed, the mixture was neutralized with 2 N H_2SO_4. The insoluble material was filtered off and the filtrate was concentrated to dryness. The residue was recrystallized from aqueous acetone. On admixture with an authentic sample,[1] the melting point (135—137°) was not depressed. The yield was 3.18 g, based on 4-nitro-2-formylpyridine ethylene glycol acetal.

4-Methoxy-2-pyridylmethanol 1-Oxide (5). Alternate Synthesis B——Sodium methoxide (10 mmol) solution was added with stirring to a solution of **4** in absolute methanol (70 ml). The solution was stirred at room temperature for 16 hr. After ascertaining by TLC (solvent system $CHCl_3$–EtOH 10: 1) that the reaction was complete, the solvent was removed *in vacuo*. The residue was recrystallized from *n*-pentane/EtOH. The yield was 0.89 g (74.4%). mp 135—137°. The reaction could be scaled-up to a 0.1 mol level without reducing the yield.

General Procedure for the Synthesis of 4-Substituted-2-picolyl Chloride 1-Oxide (7, 10, and 11)——2-Hydroxymethyl-4-substituted 1-oxide (16.4 mmol) was added in portions to freshly distilled (stirred and ice-cooled) thionyl chloride (14 ml). The temperature of the reaction mixture was allowed to rise to ambient temperature. Stirring was continued for 20 min, and the solution was then kept at 65° for ten minutes. Vigorous evolution of gas was observed. After cooling, the whole was carefully concentrated to dryness *in vacuo*. In the cases of **10** and **11**,[14] the residue was recrystallized from EtOH–AcOEt (1: 1). The residue was suspended in ethyl acetate (30 ml) containing *ca.* 0.5 ml of H_2O. Powdered $NaHCO_3$ (2.0 g) was added and the mixture was stirred vigorously overnight, then filtered. The filtrate was concentrated to dryness. The residue was extracted with $CHCl_3$ (50 ml×9). Concentration of the organic layer left a crystalline residue, which was recrystallized from a mixture of benzene and DMF.

Table III. Melting points, Yields, and Combustion Values of the Chlorides (**7**, **10**, and **11**)

Compd.	mp, °C	Yield (%)	Calcd %				Found %			
			C	H	N	Cl	C	H	N	Cl
7	104—105	79.3	38.20	2.65	14.85	18.83	38.44	2.61	14.98	18.51
10	112—113	86.2	48.43	4.64	8.07	20.42	48.41	4.57	8.00	20.38
11	137—138	83.5	44.44	4.23	7.40	18.78 S (16.93)	44.34	4.23	7.40	18.40 S (16.95)

2′-*O*-(4-Nitro-2-picolyl 1-Oxide)uridine (14)——A solution of 2′,3′-*O*-dibutylstannylene uridine (2.37 g, 5 mmol) and (4-nitro-2-picolyl 1-oxide) chloride (2.8 g, 10 mmol) in DMF (30 ml) was heated at 110° for 1 hr. The reaction was monitored by TLC (solvent system, $CHCl_3$–EtOH 5: 1). The solvent was removed *in vacuo*, and the residue was dissolved in a minimum amount of DMF, then subjected to column chromatography (silica 80 g, column size 2.6 cm×33). The column was initially washed with $CHCl_3$ (200 ml), then with $CHCl_3$–MeOH (19: 1 v/v, 1.4 l), and finally with $CHCl_3$–MeOH (15: 1 v/v, 4.4 l). Only the latter fraction (4 l) contained UV-absorbing material. The fraction containing the desired product [λ_{max} (MeOH) 260 and 330 nm; no consumption of metaperiodate] was pooled and concentrated to dryness (2.2 g). The residue was twice recrystallized from MeOH with the aid of activated charcoal, mp 225—227°. Yield, 282 mg (12%). UV λ (MeOH) nm: 330 (11000), 260 (12000). NMR (DMSO-d_6) δ: 5.98, 4.18, 3.98. *Anal.* Calcd for $C_{15}H_{16}N_3O_4$: C, 45.46; H, 4.09; N, 14.14. Found: C, 45.31; H, 4.18; N, 13.82.

13) On methoxide treatment, the absorption maximum of 5 at 330 nm shifted to 271 nm, indicating that conversion of the nitro group to a methoxy group had taken place.

14) In this procedure, **10** and **11** were obtained as the hydrochloride, whereas **7** was obtained as the free base.

2′-*O*-(4-Methoxy-2-picolyl 1-Oxide)uridine (15)——Compound (**14**) [*Rf* 0.66 in $CHCl_3$–EtOH (1: 1)] (15 mg, 0.04 mmol) was dissolved in absolute methanol (30 ml) containing methanolic sodium methoxide (0.2 ml) which had been prepared by dissolving 1.69 g of sodium in 100 ml of MeOH. The solution was kept at 60° for 15 min. The structure of the product was confirmed by UV,[13] TLC [the *Rf*-value of **15** was 0.21 (single spot) in the same solvent as above] and NMR.

2′- and 3′-*O*-(4-Methoxy-2-picolyl 1-Oxide)adenosine (17) and (18)——A solution of (4-methoxy-2-picolyl 1-oxide)chloride (1.74 g, 10 mmol) in DMF (35 ml) was slowly added to a solution of 2′,3′-*O*-(dibutylstannylene)adenosine[6] (2.5 g, 5 mmol) in DMF (50 ml), and the mixture was heated at 85°. The reaction was monitored by TLC ($CHCl_3$–EtOH 1: 1). After 18 hr, the solvent was removed. The residue (5.6 g) was dissolved in EtOH (25 ml) and subjected to thin-layer chromatography (solvent system, $CHCl_3$–EtOH 1: 1; twenty-two plates of 20 cm × 20 were used). Development was repeated twice. Seven separate bands were discernible under an ultraviolet lamp, and were designated from the top as band 1 through band 7. Band 5 was scratched off and extracted with 2 l of $CHCl_3$–EtOH (5: 1). Concentration of the extract left a residue (595 mg). A portion of the residue (300 mg) was again subjected to thin layer chromatography with the same solvent system, using eight plates of the same size. The band containing product(s) was separated. Extraction of the band with MeOH and removal of the solvent left a mixture of 2′-*O*-(**17**) and 3′-*O*-isomers (**18**) (60 mg). Recrystallization from EtOH afforded an analytical sample of the 2′-*O*-isomer (20 mg). mp 240.5—242.5°. *Anal.* Calcd for $C_{17}H_{20}N_6O_6$: C, 50.49; H, 4.98; N, 20.78. Found: C, 50.47; H, 5.02; N, 20.71.

The above mother liquor was rechromatographed as described above. After work-up the 3′-*O*-isomer (**18**) was obtained as a homogeneous foam. The signal of the anomeric proton of the 3′-*O*-isomer appeared at 5.97 ppm (d, J=6.5 Hz, 1H).

Alternative (Improved) Synthesis of 17——Well-dried adenosine (1.28 g, 4.79 mmol) was dissolved in hot DMF (43 ml) and the solution was rapidly cooled with swirling to 0°. NaH (249.7 mg, 6.24 mmol) which had been washed with benzene was added. The mixture was stirred for 1 hr at 0°, then a DMF (40 ml) solution of **10** (1.25 g, 7.2 mmol) was added dropwise during 75 min. The stirring was continued, and the reaction was followed by TLC (solvent: methanol). After acertaining that the reaction was almost complete, the mixture was neutralized with a few drops of acetic acid. The whole was concentrated *in vacuo* to *ca.* 40 ml and poured into H_2O (0.48 l). The aqueous solution was again concentrated *in vacuo* until crystals precipitated. These were collected, and recrystallization from *aq.* MeOH afforded a first crop (103.7 mg). The above filtrate was concentrated to dryness. The residue was dissolved in MeOH (1 ml) and applied to a silica gel column (silica, 13 g; column size, 1.9 × 19 cm). The column was washed with MeOH. The fractions containing **17** were pooled. Removal of the solvent and recrystallization from MeOH afforded a second crop of **17**. The combined yield was 1.09 g (56.6%). This sample was found to be identical with the above sample of **17** on the basis of *Rf* value (in MeOH), mp, and NMR spectral data.

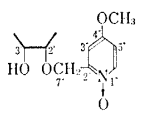
The experiment could be scaled-up to a 9 mmol (adenosine) level without reducing the yield.

2′-*O*-(4-Methylthio-2-picolyl 1-Oxide)adenosine (20)——A cooled (at 0°) solution of adenosine (161 mg, 0.6 mmol) in dry DMF (5 ml) was treated with NaH (60% in oil, 35 mg). The solution was stirred for 1 hr at 0° and a DMF (5 ml) solution of **11** (206 mg) was then added. The resulting mixture was stirred for a further 18 hr at room temperature. The solvent was then removed *in vacuo*. The residue was dissolved in MeOH containing a small quantity of H_2O. The solution was applied to a silica gel column (column size, 1 cm × 30). The column was washed with MeOH, collecting fractions of *ca.* 40 ml. The eluate was monitored by TLC (solvent MeOH). Fractions (Nos. 5—8) containing the product were pooled. The solvent was evaporated off to precipitate crystals, mp 254—256° (dec.). The yield was 93.2 mg (36.7%). NMR (DMSO-d_6) δ: 8.37 (d, J=6 Hz, $H_{6''}$),[15] 8.11 (s, 1H, H-2); 7.18—7.40 (m, 4H, NH_2, $H_{3''}$ and $H_{5''}$), 6.10 (d, J=6.1 Hz, H-1′), 4.69 (2H, J_{gem}=13 Hz), 4.08 (m, 1H, H_4'), 3.64 (s, 2H, H_5'), 2.42 (s, 3H, $-SCH_3$). *Anal.* Calcd for $C_{17}H_{20}N_6O_5S$: C, 48.57; H, 4.78; N, 19.99; S, 7.63. Found: C, 48.43; H, 4.82; N, 19.97; S, 7.57.

Deblocking of (4-Methoxy-2-picolyl 1-Oxide) Protection. Preparation of Adenosine from 17——A solution of **17** (26 mg) in acetic anhydride (4 ml) was heated at 40° for 13 hr. The reaction was monitored by TLC (solvent system: $CHCl_3$–EtOH 5: 1). After ascertaining that the rearrangement was completed, the solvent was evaporated off. The residue was co-distilled five times with H_2O (1 ml × 5). The final residue was dissolved in conc. NH_4OH (5 ml). The mixture was kept at room temperature overnight. The solvent was removed *in vacuo*. Mixed mp determination with adenosine showed no depression. Yield, 17 mg (81%).

2′-*O*-(4-Methoxy-2-picolyl 1-Oxide)adenosine 5′-Phosphate (9) and Synthesis of Adenosine 5′-Phosphate from 17——2′-*O*-(4-Methoxy-2-picolyl 1-oxide)adenosine (**17**, 217 mg, 0.54 mmol) was added with stirring to a well-stirred, cooled (at 0°) solution of phosphoryl chloride (0.1 ml) in triethyl phosphate (12.5 ml). The reaction was monitored by TLC (avicel; solvent system, *n*-PrOH–conc. NH_4OH–H_2O 20: 20: 3). After 12.5 hr, the starting material had disappeared almost completely. The whole reaction mixture was poured into dry ether (*ca.* 100 ml). Precipitates were collected by centrifugation, and dissolved in H_2O (80 ml).

15) Double prime numbering, *e.g.*, 4″-OCH_3, refers to the protecting group, and the numbering of the pyridine ring is as follows.



The solution ($TOD_{260\,nm}$: 1.29×10^4) was applied to a DEAE cellulose column (bicarbonate form: column size: 2.2×34 cm). The column was washed with a linear gradient system (0.01 M TEAE to 0.3 M TEAB), collecting fractions of 21 g. Fractions 81—96 were pooled and lyophilized to furnish a white powder. ^1H NMR showed that the signal of the 5′-proton was shifted downfield, compared with that of adenosine. $TOD_{260\,nm}$: 6450. Yield, 57.8%.

The dried powder (27 mg) was dissolved in DMF (1 ml) and acetic anhydride (0.1 ml) was added. The resulting solution was heated at 45°. The reaction was monitored by PEP (750 V, 0.05 M TEAB, 30 V/cm). The reaction was completed in 48 hr. The solvent was removed *in vacuo*. A portion was dissolved in methanol saturated with ammonia at 0°. The solution was stored at room temperature overnight. The product behaved exactly like 5′-AMP on Avicel TLC (solvent systems, isoPrOH–$(NH_4)_2SO_4$–H_2O 2: 70: 19; *n*-PrOH–conc.NH_4OH–H_2O 20: 20: 3), rather than 3′-AMP.

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