Synthesis and Properties of *N*-Phosphorylated Ribonucleosides

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Abstract: A new class of phosphorylated nucleosides, i.e., adenosine 6-*N*-phosphoramidate (6-*N*-AMP) and related derivatives, were synthesized in good yields via phosphitylation of the amino group of appropriately protected adenosine derivatives. In a similar manner, cytidine 4-*N*-phosphoramidate (4-*N*-CMP), guanosine 2-*N*-phosphoramidate (2-*N*-GMP), and their diethyl ester derivatives were synthesized. These new compounds were characterized by ^{1}H, ^{13}C, and ^{31}P NMR, UV, CD, IR, electrophoresis, and mass spectroscopy. The physical and chemical properties of these *N*-phosphorylated ribonucleoside derivatives have been studied in detail.

Introduction

Functional groups of nucleic acids are modified in a variety of ways in living cells. Such modified species play essential roles in a series of biological reactions.[1] Phosphorylation of the 5'-terminal hydroxyl of DNA/RNA is catalyzed by specific enzymes such as polynucleotide phosphorylase.[2] The 3'-terminal aminoacylation of tRNA is one of the most prevalent enzymatic reactions in peptide synthesis.[3] It is also well known that 2'-hydroxyl groups of RNA are methylated,[4] ribosylated,[5] and phosphorylated.[6] On the other hand, a large number of nucleosides modified at their nucleobases have been discovered from biologically important nucleic acids such as tRNA and mRNA.[7] For example, a wide variety of 6-*N*-modified adenosine derivatives such as 6-*N*-methyladenosine,[8] 6-*N*,6-*N*-dimethyladenosine,[8] 6-*N*-isopentenyladenosine,[9] 6-*N*-(*cis*-4-hydroxyisopentenyl)adenosine,[10] and 6-*N*-(threoninocarbonyl)adenosine (t^6A)[11,12] have been found. Many *N*-alkylated and *N*-acylated ribonucleosides have also been isolated as cytidine and guanosine derivatives.[7]

From the chemical point of view, the *exo*-amino groups of adenosine and cytidine have sufficient reactivities toward electrophiles such as acylating or phosphorylating reagents. Particularly, the cytidine base is so reactive that selective *N*-acylation without modification of other functional groups can be achieved.[13,14] Todd first reported the chemical *N*-phosphorylation of cytidine with phosphorylating reagents.[15] Letsinger has recently described a new method for oligodeoxyribonucleotide synthesis via the phosphoramidite approach by use of phosphoramidite building units having unprotected nucleobases.[16,17] The free exocyclic amino groups of deoxyadenosine and deoxycytidine proved to be partially phosphitylated with activated phosphoramidite reagents. Hayakawa described a facile *O*-selective phosphorylation of nucleosides without *N*-protection.[18] In connection with this study, he prepared an *O*-protected *N*-diethoxyphosphorylated deoxyadenosine derivative with the help of HPLC.

These results imply that such chemically reactive amino functions should also be possible sites for biological phosphorylation. However, there are no examples of naturally occurring *N*-phosphorylated nucleosides despite the sufficient chemical reactivity of nucleobases accessible to phosphorylation. Why are *N*-phosphorylated nucleosides not found from biological sources to date? Is it impossible to phosphorylate the amino group of adenosine, cytidine, or guanosine via an enzymatic process? In connection with these simple questions, there are several precedents of P–N bond containing natural products such as phosphocreatine,[19] phosphoarginine,[20] phosphoramidon,[21] and dinogunellin.[22] A more straightfoward P–N bond containing nucleoside analog is an *N*-phosphoribosylated 3'-deoxyarabinosyladenine derivative (Agrocin 84)[23,24] which was discovered as an antibiotic responsible for the biological control of crown gall by Kerr in 1977. Kerr's discovery strongly suggests that

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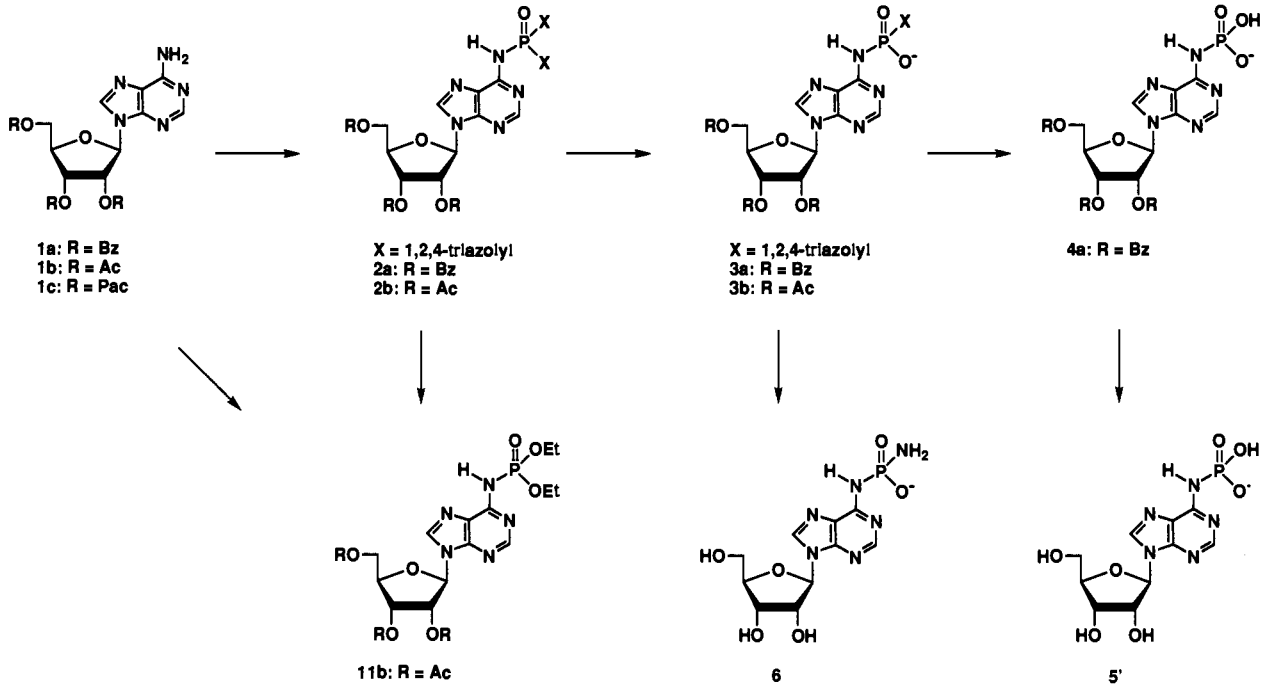
Scheme 1



N-phosphorylation could not be ruled out as a possible biological reaction. In Kerr's paper, he synthesized *N*-phosphorylated adenosine (6-*N*-AMP) as a reference compound and suggested that *N*-phosphorylated nucleosides should have phosphoryl transfer potential as putative intermediates in certain phosphorylation cycles by enzymes in living systems. However, the details of the synthesis and properties of 6-*N*-AMP were not described, and only its UV data were available.[23] It is still unknown whether the N–P bond of *N*-phosphorylated ribonucleosides is extremely unstable in living cells so that the presence of such *N*-phosphorylated species cannot be detected even if they can be formed enzymatically. In general, the inherent instability of P–N bond containing phosphoramidate derivatives has been noted.[25]

Here we have synthesized all three *N*-phosphorylated ribonucleosides involving *N*-phosphorylated cytidine (4-*N*-CMP) and guanosine (2-*N*-GMP) and 6-*N*-AMP to unveil their unknown nature. In this paper we report for the first time convenient syntheses of these *N*-phosphorylated ribonucleosides with high purity and also describe fully their chemical and physicochemical properties.

Results and Discussion

Synthesis of Adenosine 6-*N*-Phosphoramidate and Its Derivatives. Phosphorylation of a properly protected adenosine derivative with a free amino group was examined. Tris(1,2,4-triazolyl)-phosphine oxide, prepared *in situ* from phosphorus oxychloride and triazole in the presence of Et_3N, was allowed to react with 2′,3′,5′-tri-*O*-benzoyladenosine (**1a**)[26] (Scheme 1) for 2 h, and the mixture was treated successively with 0.5 M triethylammonium hydrogen carbonate. Unexpectedly, only one of the triazolyl groups of **2a** was selectively hydrolyzed to afford relatively stable triethylammonium 2′,3′,5′-tri-*O*-benzoyladenosine 6-*N*-[(1,2,4-triazolyl)phosphoramidate] (**3a**) which showed a characteristic ^{31}P NMR resonance signal at −15.3 ppm in $CDCl_3$. It was noteworthy that compound **3a** could be purified by silica gel column chromatography and isolated in 80% yield. When 2′,3′,5′-tri-*O*-acetyladenosine (**1b**)[27] was employed as a starting material in place of **1a**, the corresponding 6-*N*-[(1,2,4-triazolyl)phosphoramidate] **3b** was formed, but could not be extracted with organic solvents from aqueous solution because of its high hydrophilicity. The triazolyl group of **3a** was hydrolyzed by treatment with 80% acetic acid to give 6-*N*-phosphoramidate derivative **4a** in 89% yield (estimated by ^{31}P NMR). Unfortunately, compound **4a** gradually decomposed during removal of acetic acid by evaporation. Treatment of the crude mixture containing **4a** with aqueous ammonia afforded the desired product of 6-*N*-AMP **5′** (ammonium salt) in an overall yield of 47% from **3a** (estimated by ^{31}P NMR). When compound **3a** was treated directly with aqueous ammonia, adenosine 6-*N*-phosphorodiamidate (**6**) was obtained with a purity of 83% (estimated by ^{31}P NMR; 2.64 ppm, D_2O).

A more efficient route to 6-*N*-AMP was also explored using a highly reactive phosphoramidite, bis(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine. This reagent allowed facile *N*-phosphitylation with **1a** in the presence of 1*H*-tetrazole to give the 6-*N*-phosphitylated intermediate **7a** (Scheme 2). Compound **7a** was oxidized with *tert*-butyl hydroperoxide to give a fully protected adenosine 6-*N*-[bis(2-cyanoethyl) phosphoramidate] (**8a**). It was, however, observed that **8a** was unstable and decomposed considerably during silica gel column chromatography. After screening several solvent systems for chromatography, this problem was finally overcome by addition of 0.2% acetic acid to the usual eluent CH_2Cl_2–MeOH. Thus, compound **8a** could be isolated in pure form in a high yield of 94% without decomposition. This finding is of great importance since a route to a large scale synthesis of 6-*N*-AMP **5** is now possible. The effect of acetic acid on the stability of **8a** on the surface of silica gel is not clear. Debenzoylation of **8a** was performed by treatment with aqueous ammonia. Purification of 6-*N*-AMP **5** thus formed was done by gel filtration (Sephadex G10) followed by C18 reversed-phase column chromatography. The desired product 6-*N*-AMP **5** was obtained in 91% yield as a disodium salt[28] and

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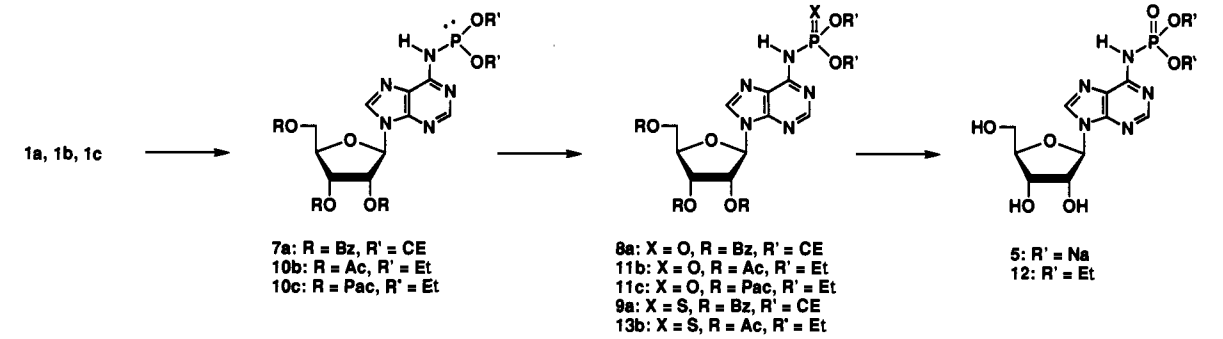
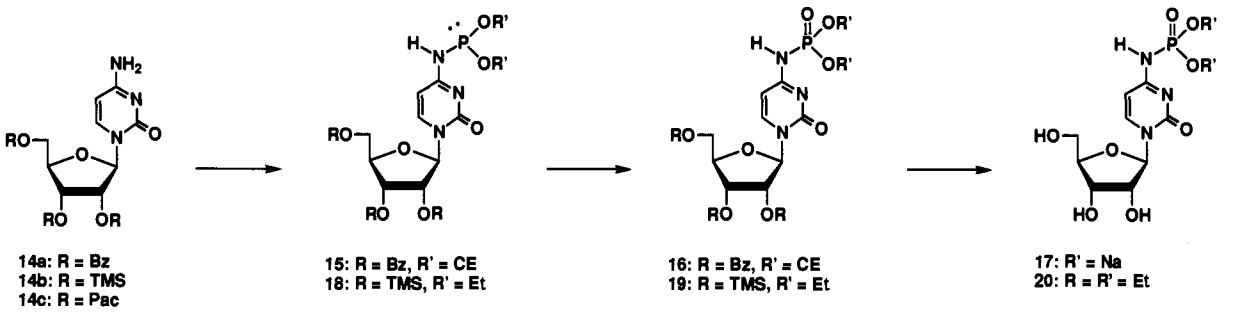
Scheme 2



Scheme 3



characterized by ^1H, ^{13}C, and ^{31}P NMR, UV, and CD spectroscopy, paper electrophoresis, HRMS spectrometry, and elementary analysis as will be discussed later. All data obtained showed that the purity of 6-*N*-AMP **5** was more than 95% (see supplementary material).

The synthesis of a phosphorothioate analog of 6-*N*-AMP was attempted. The 6-*N*-phosphitylated intermediate **7a** was treated with *N,N,N',N'*-tetraethylthiuram disulfide (TETD)[29] to give the corresponding phosphorothioamidate **9a** in 52% yield. However, deprotection of **9a** was unsuccessful when aqueous NH_3 was employed. Since the 2-cyanoethyl group of the phosphorothioate analog **9a** was more stable than that of the corresponding **8a**, the unexpected P–N bond cleavage proceeded faster than β-elimination of the 2-cyanoethyl group.

Next, the synthesis of the diethyl ester **12** of 6-*N*-AMP was examined.[30] 2',3',5'-Tri-*O*-acetyladenosine 6-*N*-[bis(1,2,4-triazolyl)phosphate] (**2b**), *in situ* prepared from **1b** with tris(1,2,4-triazolyl)phosphine oxide, was treated with excess EtOH, and the mixture was stirred for 2 h. After purification by silica gel column chromatography, the desired diethyl ester **11b** was obtained in 43% yield from **1b**. When **1b** was allowed to react with a mixture of diethyl phosphonate, carbon tetrachloride, and triethylamine, the phosphorylation proceeded very slowly and was completed in 2 days to give **11b** in 38% yield. When diethyl phosphorochloridite was used as a phosphitylating reagent, the phosphitylation of **1b** was completed within 2 h. After oxidation with *tert*-butyl hydroperoxide, **11b** was obtained in 91% yield. The phosphorothioate derivative **13b** was also obtained in 52% yield from **1b** by treatment of the common intermediate **10b** with elemental sulfur in CS_2.

The selective removal of the acetyl groups of **11b** was attempted by treatment with aqueous NH_3. However, slight degradation of the ethyl ester moiety was observed in TLC monitoring. Therefore, the phenoxyacetyl group was used in place of the acetyl group. A similar *N*-phosphitylation of 2',3',5'-Tris-*O*-(phenoxyacetyl)adenosine (**1c**) with diethoxy(*N,N*-diisopropylamino)-phosphine followed by oxidation of the intermediate **10c** with *tert*-butyl hydroperoxide gave **11c** in 81% yield. Thus, selective deacylation of **11c** could be performed under very mild conditions without decomposition of the ethyl ester moiety and afforded the diethyl ester **12** of 6-*N*-AMP in 63% yield.

Synthesis of Cytidine 4-*N*-Phosphoramidate and Its Derivatives. A two-step *N*-phosphorylation of 2',3',5'-tri-*O*-benzoylcytidine (**14a**) (Scheme 3), prepared by the selective *N*-debenzoylation of 2',3',5'-*O*-4-*N*-tetrabenzoylcytidine,[31] was carried out by the use of bis(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphine as the phosphoramidite reagent in a manner similar to that described in the case of **8a**. Thus, 4-*N*-[bis(2-cyanoethoxy)phosphoryl]-cytidine derivative **16** was synthesized in 61% yield. Deprotection of **16** with aqueous NH_3 gave cytidine 4-*N*-phosphoramidate **17** in 76% yield.

2',3',5'-Tris-*O*-(phenoxyacetyl)cytidine (**14c**) was preferable as a starting material for the synthesis of the diethyl ester **20** of 4-*N*-CMP **17**. It was, however, difficult to prepare **14c** by the selective *N*-deacylation of the corresponding peracylated derivative. Therefore, the trimethylsilyl (TMS) group was used as a transient protective group for the hydroxyl function.[32,33] Cytidine was treated with hexamethyldisilazane to give 2',3',5'-tris-*O*-(trimethylsilyl)cytidine (**14b**) quantitatively. Further one-pot reaction of **14b** with diethoxy(*N,N*-diisopropylamino)phosphine was carried out in the presence of 1*H*-tetrazole in CH_3CN. It was found that the TMS ether bonds of **14b** were sufficiently stable during 1*H*-tetrazole-mediated phosphitylation. After oxidation with *tert*-butyl hydroperoxide, the TMS group was hydrolyzed by the addition of water to give the diethyl ester **20** of 4-*N*-CMP in 75% yield from cytidine.

Synthesis of Guanosine 2-*N*-Phosphoramidate and Its Derivatives. It is well known that the amino group of guanosine is less reactive toward acylating reagents. In contrast to this fact, the lactam moiety of guanosine is reactive with acyl chlorides,[34,35]

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(31) This compound was prepared from 4-*N*-2',3',5'-*O*-tetrabenzoylcytidine by using $ZnBr_2$ as an *N*-selective debenzoylating reagent: Kierzek, R.; Ito, H.; Bhatt, R.; Itakura, K. *Tetrahedron Lett.* **1981**, *22*, 3761.

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Scheme 4

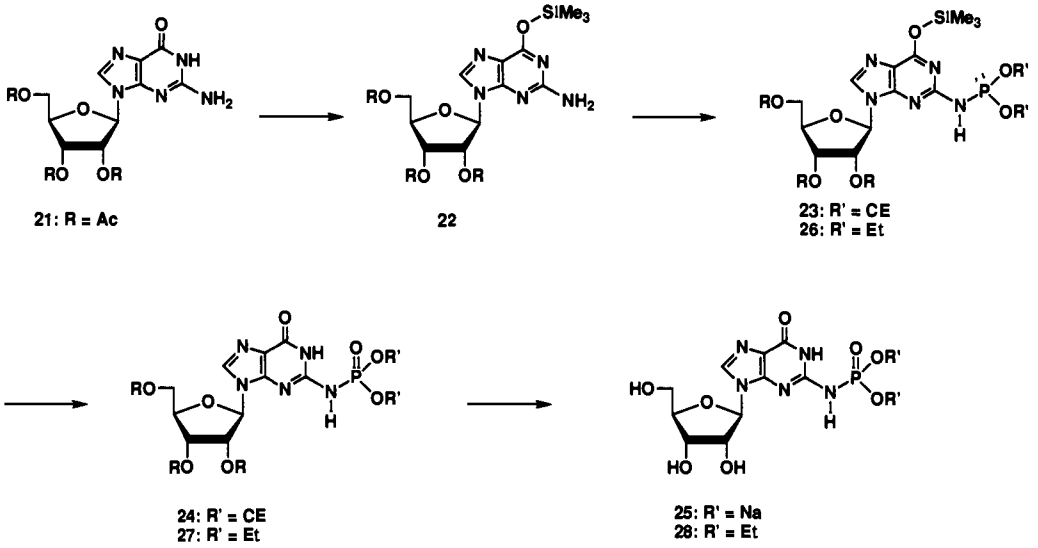

Table 1. ^{1}H NMR Chemical Shifts (ppm) of *N*-Phosphorylated Ribonucleosides in D_2O

	1′-H	2′-H	3′-H	4′-H	5′-H	5″-H	2-H	8-H	5-H	6-H
adenosine	5.99	4.74	4.38	4.25	3.87	3.78	8.14	8.26		
5′-AMP	6.04	4.77	4.46	4.35	4.01	4.01	8.12	8.55		
6-*N*-AMP **5**	6.00	4.71	4.37	4.23	3.86	3.76	8.32	8.24		
6-*N*-AMP-Et (**12**)	6.00	4.76	4.40	4.20	3.88	3.80	8.43	8.41		
cytidine	5.84	4.25	4.15	4.08	3.88	3.76			5.99	7.79
5′-CMP	6.01	4.36	4.34	4.25	4.04	3.99			6.14	8.13
4-*N*-CMP **17**	5.90	4.31	4.19	4.11	3.91	3.79			6.63	7.90
4-*N*-CMP-Et (**20**)	5.83	4.26	4.13	3.84–3.92		3.76			6.25	8.01
guanosine	5.86	4.69	4.36	4.19	3.84	3.76		7.95		
5′-GMP	5.93	4.76	4.50	4.33	4.02	4.02		8.20		
2-*N*-GMP **25**	5.88	4.70	4.38	4.18	3.86	3.78		7.96		
2-*N*-GMP-Et (**28**)	5.96	4.83	4.46	4.16	3.89	3.79		8.09		

phosphorylating reagents,[36,37] phosphitylating reagents,[38,39] sulfonyl chlorides,[36,37,40,41] and silyl chlorides[37] to give 6-*O*-modified guanosine derivatives. When 2′,3′,5′-tri-*O*-acetylguanosine (**21**)[27] was treated with an equimolar amount of diethoxy(*N*,*N*-diisopropylamino)phosphine in the presence of 1*H*-tetrazole, no reaction was observed by TLC analysis. However, ^{31}P NMR monitoring of the reaction indicated that phosphitylation occurred at the 6-*O*-position.[42] The 6-*O*-phosphitylated derivative rapidly decomposed on TLC plates, and the product could not be detected by TLC analysis. In order to protect the 6-*O*-position and increase the nucleophilicity of the amino group of guanosine, we converted **21** to the 6-*O*-TMS derivative **22** by treatment with trimethylsilyl chloride in pyridine (Scheme 4). To the resulting mixture containing **22** was added diethoxy(*N*,*N*-diisopropylamino)phosphine or bis(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine.[43] The phosphoramidite reagent was converted effectively to a more reactive phosphorus chloridite intermediate by the *in situ* reaction catalyzed by pyridinium hydrochloride generated in the initial reaction of **21** with trimethylsilyl chloride. In this case, *N*-phosphitylation proceeded smoothly to give the phosphite intermediate **23** or **26** within 1 h. Successive oxidation of **23** and **26** with *tert*-butyl hydroperoxide afforded the dialkyl esters **24** and **27** of 2-*N*-phosphorylated guanosine in 27% and 45% yields, respectively. The relatively low yields were attributed to the inherent instability of these compounds during silica gel column chromatography since TLC suggested that each reaction provided a major product to a degree of more than 80%. Treatment of the bis(2-cyanoethyl) ester **24** with aqueous NH_3 resulted in a high-yield synthesis of 2-*N*-GMP **25** in 99% yield. The diethyl ester **28** of 2-*N*-GMP was also obtained in 86% yield by the selective hydrolysis of **27**.

Characterization of *N*-Phosphorylated Ribonucleosides. ^{1}H NMR. The ^{1}H NMR spectrum of **5** (disodium salt) in D_2O basically resembles that of adenosine. The signals of the 8-H and 2-H protons are characteristic; the 8-H signal (8.24 ppm) appears at slightly higher field than the 2-H signal (8.32 ppm). The assignment of these signals was confirmed by the observation of the strong NOE between the 8-H and 1′-H protons. The large downfield shift of the 8-H proton may be attributed to the electron-withdrawing effect of the 6-*N*-phosphoryl group. Further, the acidity of the 8-H proton is enhanced for the same reason. In fact, the 8-H proton underwent gradual deuterium exchange in D_2O. A similar tendency was observed for the diethyl ester derivative **12**. The chemical shifts of 6-*N*-phosphorylated adenosine derivatives are summarized in Table 1 along with those of adenosine and 5′-AMP as reference compounds.

In the case of 4-*N*-phosphorylated cytidine derivatives, the chemical shifts of the sugar protons were similar to those of cytidine (Table 1). Characteristic features of the ^{1}H NMR spectra of **17** and **20** were the large downfield shifts of the 5-H and 6-H protons. The same effect has been cited for 4-*N*-acylated cytidine derivatives.[44]

In contrast to the above case, 2-*N*-phosphorylated guanosine

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Table 2. ^1H–^1H Coupling Constants (Hz) of *N*-Phosphorylated Ribonucleosides in D_2O

	1′, 2′	2′, 3′	3′, 4′	4′, 5′	4′, 5″	5′, 5″
adenosine	6.3	5.3	3.3	2.6	3.6	−12.9
5′-AMP[a]	5.9	5.0	3.6	3.2	3.2	*b*
6-*N*-AMP **5**	5.9	5.0	3.6	2.6	3.3	−12.9
6-*N*-AMP-Et (**12**)	5.9	5.0	3.6	2.6	3.6	−12.9
cytidine	4.0	4.3	5.9	2.6	4.3	−12.9
5′-CMP[a]	4.4	4.5	4.6	2.5	2.9	−12.0
4-*N*-CMP **17**	3.6	5.3	6.3	2.8	4.6	−12.9
4-*N*-CMP-Et (**20**)	3.6	4.6	*b*	*b*	4.0	−12.9
guanosine	5.8	5.6	4.0	3.0	4.0	−12.9
5′-GMP[a]	6.0	5.0	3.7	3.4	3.4	*b*
2-*N*-GMP **25**	5.3	5.6	4.3	3.0	3.3	−12.9
2-*N*-GMP-Et (**28**)	5.0	5.3	5.3	3.6	5.3	−12.5

[a] See ref 49. [b] Individual coupling constants could not be determined.

derivatives **25** and **28** showed ^1H NMR spectra approximate to that of guanosine even for the guanine base protons (Table 1). This indicates that there were no significant effects of the 2-*N*-phosphoryl group on the chemical shifts of the guanine base protons.

Conformation of Ribose Residues Concerning the Ratio of 2′-C- and 3′-C-*endo* Conformers. The effect of the phosphoryl group of *N*-phosphorylated ribonucleosides on the conformation of the ribose residues was examined. The proton–proton coupling constants of *N*-phosphorylated nucleoside derivatives are summarized in Table 2 along with those of the corresponding nucleosides and nucleoside 5′-monophosphates as reference compounds. In general, the $J_{1',2'}$ value of the ribose moiety reflects the ratio of 2′-C-*endo* to 3′-C-*endo* forms which is calculated by the following equation: [2′-C-*endo*] (%) = $J_{1',2'}/(J_{1',2'} + J_{3',4'}) \times 100$, where $J_{1',2'} + J_{3',4'}$ is nearly 10 Hz in general.[45] The $J_{1',2'}$ values of **5** and **12** in D_2O were 5.9 Hz each. The $J_{1',2'}$ values of adenosine were reported to be 5.8 Hz[46] and 6.1 Hz,[47] and that of adenosine 5′-monophosphate was reported to be 5.9 Hz.[48] Therefore, the ribose puckering of the *N*-phosphorylated adenosines **5** and **12** is unaffected by the presence of a phosphate or a diethoxyphosphoryl group. The $J_{1',2'}$ values of the *N*-phosphorylated guanosine derivatives **25** and **28** in D_2O were 5.3 and 5.0, respectively. The reported $J_{1',2'}$ values of guanosine, guanosine 2′-phosphate, and guanosine 5′-phosphate were 5.8, 5.2, and 6.0 Hz, respectively.[48,49] These data suggested that the *N*-phosphoryl group leads to a small shift to the 3′-C-*endo* conformer to the same degree as the 2′-phosphate group. On the other hand, the $J_{1',2'}$ values of the *N*-phosphorylated cytidine derivatives **17** and **20** were 3.6 Hz each. The $J_{1',2'}$ values of cytidine and cytidine 5′-phosphate in D_2O were 4.0 and 4.1 Hz, respectively.[49] It is apparently concluded that the $J_{1',2'}$ value of the 3′-C-*endo* conformer was increased by approximately 0.5 Hz by addition of the *N*-phosphoryl or diethoxyphosphoryl group. This result is interesting since Yokoyama reported that the *N*-acetyl group attached to 2′-*O*-methylcytidine has a significant effect on the ratio of two conformers in favor of the 3′-C-*endo* conformer.[44] This may be due to the antibonding lobe of the π^* orbital at 6-C of the 5–6 double bond contributing more effectively to intramolecular interaction with the lobe in which the lone pair electrons of the furanose ring are present.[44]

^{13}C NMR and ^{31}P NMR. The ^{13}C NMR chemical shifts of the *N*-phosphorylated ribonucleoside derivatives are summarized in Table 3 along with those of the corresponding nucleosides and nucleoside 5′-monophosphates as reference compounds.[50,51] In general, there were no significant effects of the *N*-phosphoryl group on the chemical shifts of the sugar carbons. In the case of *N*-phosphorylated guanosine derivatives **25** and **28**, the resonance signal of 2-C was observed as a doublet which was due to the P–C coupling between the phosphorus and the carbon through two bonds. However, such P–C couplings were not observed in the case of **5**, **12**, **17**, and **20**.

The ^{31}P NMR spectrum of each of the *N*-phosphorylated ribonucleoside derivatives showed the sole signal in the region around −3 to +4 ppm. Generally, the signals of the diethyl ester derivatives appeared in the upfield region.

UV Spectroscopy. The ultraviolet spectra of *N*-phosphorylated ribonucleosides (**5**, **17**, and **25**) and their diethyl esters (**12**, **20**, and **28**) at three different pH regions are shown in Figure 1 and Table 4. The pH profiles of the three *N*-phosphorylated ribonucleosides **5**, **17**, and **25** are essentially similar to those of the corresponding parent ribonucleosides. Particularly in the case of the guanosine derivative **25**, the λ_{max} values at the three pH regions are preserved but shoulders around 267 nm, which are observed in the case of guanosine, are considerably weakened. However, the λ_{max} peak of the adenosine derivative **5** at pH 4 is shifted to longer wavelength by 6–7 nm compared to that of adenosine. The intensity of **5** at λ_{max} varied remarkably at pH 7 and 13. The ratio of the ϵ values of **5** at pH 13 and 7 is 1.29. Contrary to this fact, adenosine has relatively constant ϵ values (±5%) at a wide range of pH (1.5–14). In the case of cytidine, the λ_{max} peak of **17** at pH 7 is shifted to longer wavelength by 3 nm compared with that of cytidine. The UV spectra of 4-*N*-acetylcytidine in acidic, neutral, and alkaline solutions are quite different from those of **17**.[52] These results suggest that the phosphate group on the nitrogen of the bases served not as an acyl type of group but as a substituent similar to an alkyl group like a methyl group.

CD Spectroscopy. The circular dichroism spectra of *N*-phosphorylated ribonucleosides are shown in Figure 2. It is well established that CD spectroscopy of nucleic acid monomers is particularly sensitive to the conformation around the glycosyl bond.[53] The CD spectra of 6-*N*-phosphorylated adenosine derivatives **5** and **12** were similar to those of adenosine.[53] These spectra showed the positive and negative Cotton effects around 270 and 220 nm, respectively (Figure 2A,B). These results suggested the 6-*N*-phosphorylated adenosine derivatives have the same *syn* conformation around the glycosyl bond as adenosine and 5′-AMP.[53] In the case of the diethyl ester **12**, a relatively strong positive Cotton effect was observed around 235 nm which was considerably weak in 6-*N*-AMP **5**.

In the case of 4-*N*-phosphorylated cytidine derivatives **17** and **20**, the CD spectra showed the negative and positive Cotton effects around 220 and 275 nm, respectively (Figure 2C,D). These spectra are similar to those of cytidine and 5′-CMP.[53] Particularly, in the case of the diethyl ester **20**, a very weak positive Cotton effect was observed as a shoulder around 240 nm.

The CD spectra of 2-*N*-phosphorylated guanosine derivatives showed strong positive and weak negative Cotton effects around 215 and 250 nm, respectively (Figure 2E,F). Guanosine and 5′-GMP have the same Cotton effects around a similar wavelength range.[53]

Generally, the above results suggest that the *N*-phosphoryl group did not affect the orientation of the purine and pyrimidine bases. The CD spectral data of the *N*-phophorylated ribonucleosides are summarized in Table 5.

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Table 3. ^{13}C NMR Chemical Shifts (ppm) of *N*-Phosphorylated Ribonucleosides

	1'-C	2'-C	3'-C	4'-C	5'-C	2-C	4-C	5-C	6-C	8-C	solvent
adenosine[a]	88.2	73.7	70.9	86.2	61.9	152.6	149.3	119.6	156.3	140.2	DMSO
5'-AMP[b]	87.0	70.5	74.5	84.2	63.5	152.4	148.4	118.0	155.0	139.9	H_2O
6-*N*-AMP **5**	91.1	76.3	73.3	88.4	64.2	155.4	150.9	123.1	157.2	143.0	D_2O
6-*N*-AMP-Et (**12**)	91.1	76.5	73.2	88.5	64.1	154.2	152.8	125.0	154.5	145.2	D_2O
cytidine[a]	90.1	70.6	75.1	85.3	61.9	156.9	166.7	95.7	142.8		DMSO
5'-CMP[b]	89.1	69.7	74.3	83.3	63.3	157.5	166.2	95.6	141.8		H_2O
4-*N*-CMP **17**	93.1	71.9	76.6	86.4	63.5	160.2	*c*	99.7	144.5		D_2O
4-*N*-CMP-Et (**20**)	93.3	71.8	76.9	86.7	63.2	157.1	166.0	101.0	145.7		D_2O
guanosine[a]	87.3	71.5	74.9	86.4	62.2	154.6	152.3	117.5	157.8	136.9	DMSO
5'-GMP[b]	87.0	70.6	74.3	84.1	63.9	153.6	151.0	115.7	158.3	137.3	H_2O
2-*N*-GMP **25**	90.5	73.0	76.3	87.8	64.0	154.1	155.3	119.5	161.2	140.6	D_2O
2-*N*-GMP-Et (**28**)	91.1	72.7	76.0	87.4	64.1	151.4	152.6	124.1	161.4	142.2	D_2O

[a] See ref 50. [b] See ref 51. [c] Chemical shift could not be determined.

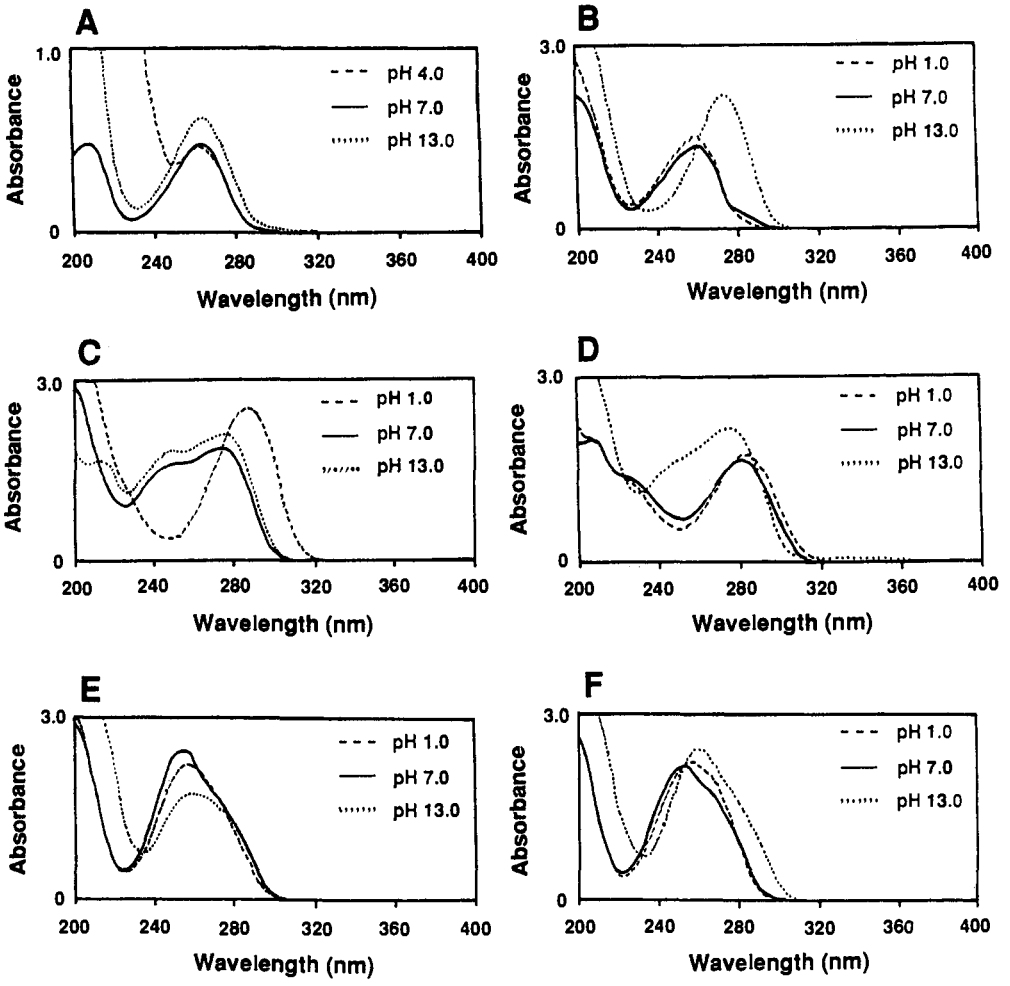

Figure 1. UV spectra of *N*-phosphorylated ribonucleosides at three different pH regions: (A) 6-*N*-AMP **5**, (B) 6-*N*-AMP-Et (**12**), (C) 4-*N*-CMP **17**, (D) 4-*N*-CMP-Et (**20**), (E) 2-*N*-GMP **25**, (F) 2-*N*-GMP-Et (**28**).

Infrared Spectroscopy. Next, the IR spectroscopic studies of *N*-phosphorylated ribonucleoside were examined. Kerr suggested that in the IR spectrum of Agrocin 84 a band at 1225 cm^{-1} is due to the P–O stretching vibration of the phosphoramidate group.[24] On the other hand, Hatano reported the IR spectrum of dinogunellin, a P–N bond containing toxic phospholipid, showing that a band around 820–1040 cm^{-1} is due to the P–N stretching vibration of the phosphoramidate group.[22] In the case of 6-*N*-AMP **5**, the IR spectra exhibited a strong band at 980 cm^{-1} which may correspond to the P–N stretching vibration of the phosphoramidate group. It is noteworthy that the band at about 1650 cm^{-1}, due to the N–H deformation vibration of the amino group of adenine,[54] was considerably weakened in the 6-*N*-phosphorylated adenosine derivatives **5** and **12**. In the case of 4-*N*-phosphorylated cytidine derivatives **17** and **20**, the bands at about 1645 cm^{-1} which are due to the N–H deformation vibration of the amino group of cytosine[61] were also weakened in the 4-*N*-phsphorylated cytidine derivatives. However, the absorption bands corresponding to the P–N stretching vibration of the phosphoramidate group of these compounds could not be identified because these bands overapped with other strong bands. In the case of 2-*N*-phosphorylated guanosine derivatives **25** and **28**, a characteristic band was observed around 960–965 cm^{-1} which may be due to the P-N or P-O stretching vibration of the phosphoramidate group.

Paper Electrophoresis. Paper electrophoresis of *N*-phosphorylated ribonucleosides and related compounds was performed at various pH regions. These results are shown in Figure 3. Adenosine derivatives behave in a manner similar to that of cytidine at a whole range of pH 3–10. At lower pH values of 3 and 3.5, *N*-phosphorylated species **5** and **17** moved remarkably

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Table 4. UV Spectral Data of *N*-Phosphorylated Ribonucleosides

	pH	λ_{max} (nm)	$\epsilon_{max} \times 10^{-3}$	λ_{min} (nm)	$\epsilon_{min} \times 10^{-3}$
6-*N*-AMP **5**	4.0	263.0	15.9	249.0	12.2
	7.0	263.0	16.6	228.5	2.5
	13.0	264.5	21.4	230.0	4.7
6-*N*-AMP-Et (**12**)	1.0	259.5	12.9	226.5	3.3
	7.0	259.5	11.5	226.5	2.6
	13.0	273.5	18.4	235.0	2.3
4-*N*-CMP **17**	1.0	286.5	12.8	247.5	1.8
	7.0	274.0	9.6	226.0	4.7
	13.0	275.0	10.8	226.5	5.7
4-*N*-CMP-Et (**20**)	1.0	283.0	7.6	250.0	2.4
	7.0	280.5	7.1	251.0	3.0
	13.0	275.5	9.4	230.0	4.8
2-*N*-GMP **25**	1.0	256.0	11.1	225.5	2.3
	7.0	254.0	12.1	224.5	2.4
	13.0	258.0	8.8	233.0	3.7
2-*N*-GMP-Et (**28**)	1.0	255.5	11.8	222.5	2.0
	7.0	253.0	11.4	221.5	2.3
	13.0	260.5	12.9	232.5	3.8

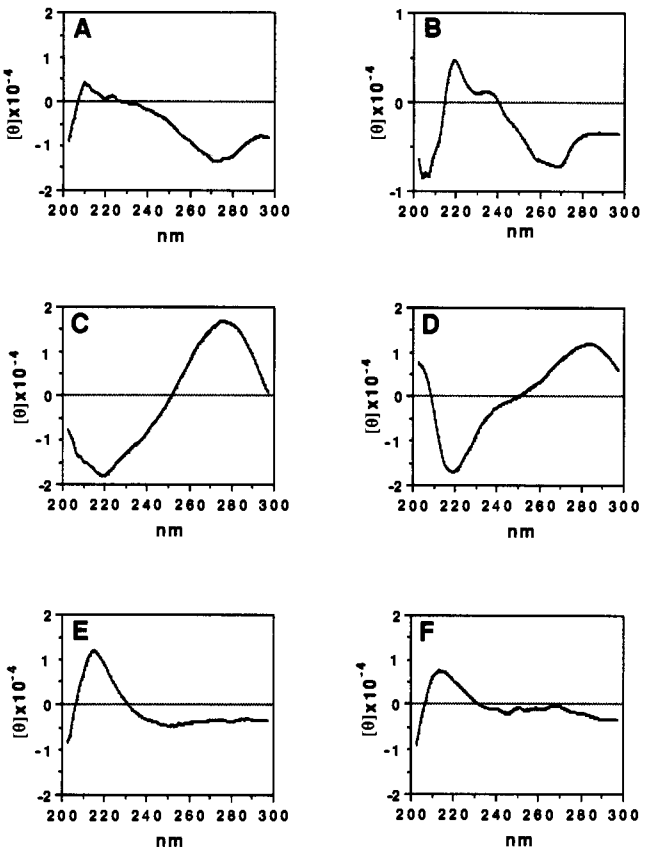

Figure 2. CD spectra of *N*-phosphorylated ribonucleosides: (A) 6-*N*-AMP **5** at pH 7.0, (B) 6-*N*-AMP-Et (**12**) at pH 7.0, (C) 4-*N*-CMP **17** at pH 7.0, (D) 4-*N*-CMP-Et (**20**) at pH 6.2, (E) 2-*N*-GMP **25** at pH 6.6, (F) 2-*N*-GMP-Et (**28**) at pH 6.0.

Table 5. CD Spectral Data of *N*-Phosphorylated Ribonucleosides

	λ_1 (nm)	$[\theta]_{\lambda_1} \times 10^{-4}$	λ_2 (nm)	$[\theta]_{\lambda_1} \times 10^{-4}$
6-*N*-AMP **5**	271.2	−1.36	210.4	0.41
6-*N*-AMP-Et (**12**)	265.8	−0.72	219.6	0.47
4-*N*-CMP **17**	275.8	1.68	219.6	−1.82
4-*N*-CMP-Et (**20**)	276.4	1.19	218.8	−1.71
2-*N*-GMP **25**	250.8	−0.48	251.2	1.19
2-*N*-GMP-Et (**28**)	246.6	−0.21	213.6	0.75

to the anode compared to pA and pC, while adenosine and cytidine moved in the reverse direction. These results indicate that **5** and **17** are not protonated in these pH regions. Interestingly, we observed that the diethyl ester **28** moved slowly toward the anode, showing a mobility of 0.24 relative to pG. Under these conditions, guanosine did not move significantly from the origin. This is

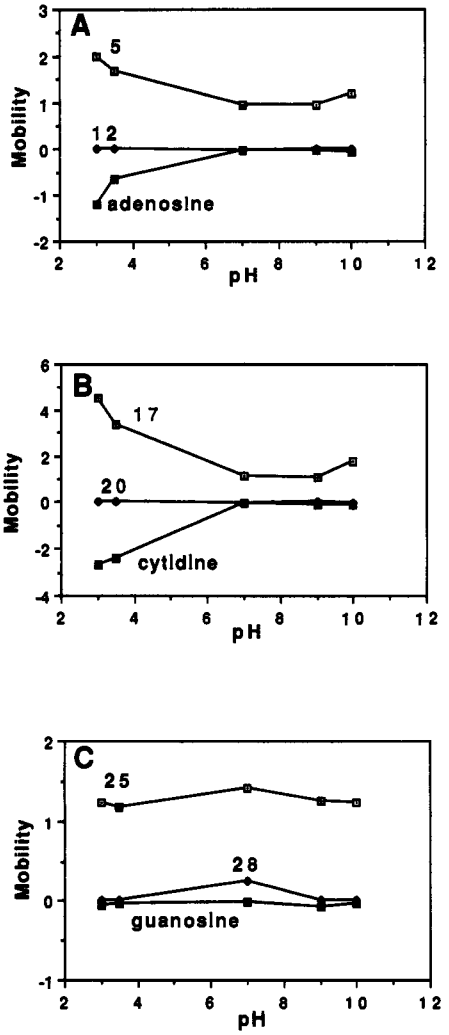

Figure 3. Paper electrophoresis of *N*-phosphorylated ribonucleosides at different pH regions: (A) adenosine, 6-*N*-AMP **5**, and 6-*N*-AMP-Et (**12**), (B) cytidine, 4-*N*-CMP **17**, and 4-*N*-CMP-Et (**20**), (C) guanosine, 2-*N*-GMP **25**, and 2-*N*-GMP-Et (**28**). The mobilities of the compounds were estimated relative to those of the corresponding parent nucleoside 5′-monophosphates as 1.0.

ascribed to partial dissociation of either the 2-phosphorylated NH group or the lactam NH at position 3 at pH 7.

Evidence of the presence of the 2′,3′-*cis*-diol groups in **5** and **12** was obtained by paper electrophoresis in a borate buffer (pH 9.0). The neutral nucleoside **12** and adenosine comigrated with each other. In a similar manner, **5** and pA comigrated in proportion to the mobilities observed at pH 9 (phosphate buffer) (data not shown).

Solubility. *N*-Phosphorylated compounds are almost freely soluble in water. This is one of the characteristic features of *N*-phosphorylated ribonucleotides. This is in clear contrast to the fact that cytidine 5′-phosphate has a poor solubility in water and guanosine 5′-monophosphate tends to cause self-aggregation in water. Even *N*-diethoxyphosphorylated ribonucleosides **12**, **20**, and **28** were also soluble in water. On the other hand, the corresponding *N*-acetylated ribonucleosides have less solubility in water so that they are often recrystallized from water. The great solubility of *N*-phosphorylated ribonucleosides in water is explained by the $P(O)P(OH)_2$ or $P(O)(OEt)_2$ function increasing the hydrophilicity due to sites accessible to hydrogen bonding with water molecules.

Stability of *N*-Phosphorylated Ribonucleosides. In general, *N*-phosphorylated ribonucleosides **5**, **12**, **17**, **20**, **25**, and **28** were quite stable under basic conditions such as 0.1 M NaOH and concentrated NH_3 for several days. On the contrary, compounds

Table 6. Stability of *N*-Phosphorylated Ribonucleosides[a]

	6-*N*-AMP **5**		6-*N*-AMP-Et (**12**)		4-*N*-CMP **17**		4-*N*-CMP-Et (**20**)		2-*N*-GMP **25**		2-*N*-GMP-Et (**28**)	
	$t_{1/2}$	t_{comp}	$t_{1/2}$	t_{comp}	$t_{1/2}$	t_{comp}	$t_{1/2}$	t_{comp}	$t_{1/2}$	t_{comp}	$t_{1/2}$	t_{comp}
0.1 M HCl	4 h	24 h	stable		8 h	3 d	8 h	3 d	5 h	30 h	8 h	3 d
80% AcOH	24 h	4 d	stable		4 d		stable		7 h	60 h	15 h	
0.1 M NaOH	stable		stable		stable		stable		stable		stable	
concd NH_3	stable		stable		stable		stable		stable		stable	

[a] All reactions were carried out at room temperature.

5, **17**, and **25** gradually decomposed in 0.1 M HCl. The diethyl esters **12**, **20**, and **28** were more stable than the corresponding unesterified phosphoramidates both in 0.1 M HCl and in 80% acetic acid. These results are summarized in Table 6. Compound **5** is sufficiently stable in 0.1 M ammonium acetate. However, the reversed-phase HPLC profile always exhibited two peaks corresponding to adenosine and **5** in a ca. 1:1 ratio. Therefore, it was impossible to monitor the reaction or stability of **5** by reversed-phase HPLC using ammonium acetate buffer.

Conclusion

The present method for the synthesis of a new class of phosphorylated nucleosides, adenosine 6-*N*-phosphoramidate (6-*N*-AMP), cytidine 4-*N*-phosphoramidate (4-*N*-CMP), guanosine 2-*N*-phosphoramidate (2-*N*-GMP), and their diethyl ester derivatives enabled us to obtain these compounds on a large scale with high purity. The new *N*-phosphorylated ribonucleosides prepared here would be useful as various kinds of substrates such as inhibitors in certain enzyme reactions or phosphoryl transfer reactions. We have obtained preliminary results of antivirus activities and mutagenicity by *N*-phosphorylated ribonucleosides. These results will be reported elsewhere. Further, the present method for the preparation of the *N*-phosphorylated nucleosides can be applied to the introduction of various functional groups such as reporter groups, photoreactive reagents, intercalators, DNA cleaving reagents, etc. via the *N*-phosphoryl group to the adenine, cytosine, or guanine base. These functionalized nucleosides can be incorporated into the specific site of oligonucleotides by chemical synthesis. The present results concerning the stability of the *N*-phosphorylated nucleosides suggested that the *N*-phosphoryl groups can be removed under acidic conditions. Therefore, it is possible to regenerate the amino group from the functionalized phosphoryl groups. The unique characteristics of the *N*-phosphorylated nucleosides would enable development of a wide variety of applications in nucleic acid chemistry.

Experimental Section

General Procedures. CH_2Cl_2 and MeCN were distilled from CaH_2 after being refluxed for several hours, and stored over molecular sieves 4A. Pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled from CaH_2, and stored over molecular sieves 4A. *tert*-Butyl hydroperoxide (containing 20% di-*tert*-butyl peroxide) was purchased from Merck & Co. Inc. *N,N,N',N'*-Tetraethylthiuram disulfide was purchased from Tokyo Kasei Inc. ^{1}H NMR spectra were obtained at 270 MHz on a JEOL-EX-270 spectrometer with tetramethylsilane as an internal standard in $CDCl_3$ and with sodium 3-(trimethylsilyl)propanesulfonate as an external standard in D_2O. ^{13}C NMR spectra were obtained at 67.8 MHz on a JEOL-EX-270 spectrometer with tetramethylsilane as an internal standard. ^{31}P NMR spectra were obtained at 109.25 MHz on a JEOL-EX-270 spectrometer using 85% H_3PO_4 as an external standard. UV spectra were recorded on a Hitachi 220A spectrophotometer. IR spectra were obtained on a Hitachi 260-50. CD spectra were obtained on a JASCO J-500C spectrometer at concentrations of 0.8–1.5 mM in water. Paper electrophoreses were carried out using 0.075 M morpholinium acetate buffer (pH 3.5) at 1500 V for 1.5 h, 0.05 M ammonium acetate buffer (pH 7.0) at 1200 V for 1.3 h, 0.1 M Tris–HCl buffer (pH 9.0) at 1500 V for 1.5 h, boric acid buffer (pH 9.0) at 1200 V for 1 h, and 0.05 M $NaHCO_3$ buffer (pH 10.0) at 1200 V for 1.5 h. The mobilities of the compounds were calculated relative to those of the corresponding parent nucleoside 5'-monophosphates. Thin-layer chromatography was performed on precoated glass plates of Kieselgel 60 F_{254} (Merck, No. 5715). Silica gel column chromatography was carried out using Wakogel C-200. Revered-phase column chromatography was performed using Waters Prep-Pak C18.

2',3',5'-Tri-*O*-benzoyladenosine (1a). Adenosine (0.535 g, 2 mmol) was dried by repeated coevaporation with dry pyridine and finally suspended in dry pyridine (20 mL). To the suspension were added benzoic anhydride (2.26 g, 10 mmol) and 4-(*N,N*-dimethylamino)pyridine (0.061 g, 0.05 mmol), and the mixture was stirred at room temperature (rt) for 2 h. The mixture was concentrated to half-volume, diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$ (aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column. Chromatography was performed with CH_2Cl_2, applying a gradient of methanol (1–1.5%). The fractions containing **1a** were combined and concentrated to give **1a** (1.136 g, 98%) as a colorless foam. ^{1}H and ^{13}C NMR spectra of this product were identical to those of the authentic material.[27]

2',3',5'-Tris-*O*-(phenoxyacetyl)adenosine (1c). Adenosine (0.267 g, 1 mmol) was dried by repeated coevaporation with dry pyridine and finally suspended in dry pyridine (10 mL). To the suspension was added phenoxyacetic anhydride (1.43 g, 5 mmol), and the mixture was stirred at rt for 10 min. The reaction was quenched with water, and the mixture was concentrated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed three times with 5% $NaHCO_3$ (aq), and the aqueous layer was back-extracted with CH_2Cl_2. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was applied to a silica gel column. Chromatography was performed with CH_2Cl_2, applying a gradient of methanol (1.5–2.5%). The fractions containing **1c** were combined and concentrated to give **1c** (0.599 g, 89%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 4.41–4.69 (9H, m, CH_2 of Pac, 4'-H, 5'-H, and 5''-H), 5.76 (2H, br s, NH_2), 5.83 (1H, dd, $J_{3',4'}$ = 4.3 Hz, $J_{3',2'}$ = 5.6 Hz, 3'-H), 6.06 (1H, d, $J_{1',2'}$ = 5.6 Hz, 1-H), 6.15 (1H, dd, $J_{2',1'} = J_{2',3'}$ = 5.6 Hz, 2'-H), 6.78–7.03 (9H, m, 3,4,5-H of Ph), 7.19–7.33 (6H, m, 2,6-H of Ph), 7.88 (1H, s, 2-H), 8.33 (1H, s, 8-H); ^{13}C NMR ($CDCl_3$) δ 63.4 (5'-C), 64.6, 64.7, 65.0 (CH_2 of Pac), 71.3 (3'-C), 73.2 (2'-C), 79.8 (4'-C), 85.8 (1'-C), 114.4, 114.5, 114.5 (2,6-C of Ph), 119.8 (5-C), 121.9, 121.9, 122.0 (4-C of Ph), 129.5, 129.5, 129.6 (3,5-C of Ph), 139.0 (8-C), 149.4 (4-C), 153.0 (2-C), 155.7 (6-C), 157.3, 157.3, 157.4 (1-C of Ph), 167.7, 168.0, 168.4 (C=O of Pac). Anal. Calcd for $C_{34}H_{31}N_5O_{10}\cdot{}^1/_5H_2O$: C, 60.66; H, 4.70; N, 10.40. Found: C, 60.60; H, 4.80; N, 10.56.

Triethylammonium 2',3',5'-Tri-*O*-benzoyladenosine 6-*N*-[(1,2,4-Triazolyl)phosphoramidate] (3a). To a mixture of 1,2,4-triazole (0.622 g, 9 mmol, dried by repeated coevaporation with dry pyridine and MeCN) and triethylamine (1.12 mL, 8 mmol) in dry MeCN (50 mL) at 0 °C was added phosphorus oxychloride (0.186 mL, 2 mmol). The reaction mixture was stirred at rt for 30 min. To the mixture was added 2',3',5'-tri-*O*-benzoyladenosine (**1a**) (0.58 g, 1 mmol, dried by repeated coevaporation with dry pyridine) in dry MeCN (10 mL). After being stirred for 2 h, the mixture was treated with 0.5 M triethylammonium hydrogen carbonate and stirred at rt for an additional 1 h. The mixture was concentrated to a small volume, diluted with $CHCl_3$, and washed six times with 0.5 M triethylammonium hydrogencarbonate, and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column. Elution was performed with CH_2Cl_2 containing 2% triethylamine, applying a gradient of methanol (0–7%). The fractions containing **3a** were combined and concentrated to dryness. The residue was dissolved in $CHCl_3$ and washed with water. The organic layer was dried over Na_2SO_4, filtered, and concentrated to give **3a** (0.65 g, 80%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ −15.3; ^{1}H NMR ($CDCl_3$) δ 1.29 (9H, t, J = 7.3 Hz, CH_3 of Et_3NH^+), 3.08 (6H, q, J = 7.3 Hz, CH_2 of Et_3NH^+), 4.6–4.9 (3H, m,

4′-H, 5′-H, and 5″-H), 6.20 (1H, dd, $J_{3',4'}$ = 4.6 Hz, $J_{3',2'}$ = 5.6 Hz, 3′-H), 6.32 (1H, dd, $J_{2',3'}$ = 5.6 Hz, $J_{2',1'}$ = 5.3 Hz, 2′-H) 6.43 (1H, d, $J_{1',2'}$ = 5.3 Hz, 1′-H), 7.32–7.59 (11H, m, ArH), 7.89–8.12 (9H, m, ArH), 8.37 (1H, s, 8-H), 8.95 (1H, s, NH); ^{13}C NMR ($CDCl_3$) δ 8.6 (CH_3 of Et_3NH^+), 45.8 (CH_2 of Et_3NH^+), 63.5 (5′-C), 71.3 (3′-C), 73.8 (2′-C), 80.5 (4′-C), 86.5 (1′-C), 121.8 (5-C), 128.1, 128.4, 128.6, 128.9, 129.2, 129.6, 129.7, 129.7, 133.2, 133.5, 133.6 (Bz), 149.8, 149.9 (triazole), 150.0 (4-C), 152.7 (2-C), 152.9 (6-C), 164.9, 165.2, 166.0 (C=O of Bz).

Triethylammonium Adenosine 6-*N*-Phosphorodiamidate (6). Triethylammonium 2′,3′,5′-tri-*O*-benzoyladenosine 6-*N*-[(1,2,4-triazolyl)phosphoramidate] (**3a**) (0.152 g, 0.2 mmol) was treated with concentrated NH_3–pyridine (9:1, v/v, 50 mL) at rt for 6 h. Ammonia and pyridine were removed by evaporation, and the residue was dissolved in water and washed five times with ether. The aqueous layer was concentrated to a small volume and applied to a column of anion exchange resin (Sephadex A25, HCO_3^- form, 80 × 20 mm). Chromatography was performed with a gradient of triethylammonium hydrogen carbonate (0–1 M), and the fractions containing **6** were combined and concentrated. Triethylammonium hydrogen carbonate was removed by repeated coevaporation with water, and the solution was lyophilized to give **6** (0.091 g, 83%) as a white powder: ^{31}P NMR (D_2O) δ 2.64; ^{1}H NMR (D_2O) δ 1.08 (9H, t, J = 7.3 Hz, CH_3 of Et_3NH^+), 2.88 (6H, q, J = 7.3 Hz, CH_2 of Et_3NH^+), 3.73 (1H, dd, $J_{5'',5'}$ = 12.9 Hz, $J_{5'',4'}$ = 3.6 Hz, 5″-H), 3.82 (1H, dd, $J_{5',5''}$ = 12.9 Hz, $J_{5',4'}$ = 2.6 Hz, 5′-H), 4.18 (1H, d, $J_{4',3'}$ = 3.3 Hz, 4′-H), 4.32 (1H, dd, $J_{3',4'}$ = 3.3 Hz, $J_{3',2'}$ = 5.3 Hz, 3′-H), 4.66 (1H, dd, $J_{2',1'}$ = 5.9 Hz, $J_{2',3'}$ = 5.3 Hz, 2′-H) 5.95 (1H, d, $J_{1',2'}$ = 5.9 Hz, 1′-H), 8.24 (1H, s, 8-H), 8.32 (1H, s, 2-H); ^{13}C NMR (D_2O) δ 11.4 (CH_3 of Et_3NH^+), 48.8 (CH_2 of Et_3NH^+), 64.1 (5′-C), 73.3 (3′-C), 76.4 (2′-C), 88.4 (4′-C), 91.0 (1′-C), 123.2 (d, J_{PNCC} = 8.6 Hz, 5-C), 143.6 (8-C), 151.4 (4-C), 155.0 (2-C), 156.2 (6-C).

2′,3′,5′-Tri-*O*-benzoyladenosine 6-*N*-[*O,O*-Bis(2-cyanoethyl)phosphoramidate] (8a). 2′,3′,5′-Tri-*O*-benzoyladenosine (**1a**) (2.318 g, 4 mmol) and 1*H*-tetrazole (0.631 g, 9 mmol) were dried by repeated coevaporation with dry pyridine and dry CH_2Cl_2 and finally dissolved in dry CH_2Cl_2 (40 mL). To the mixture was added bis(2-cyanoethoxy)(*N,N*-diisopropylamino)phosphine (1.628 g, 6 mmol). After being stirred at rt for 1 h, the mixture was treated with *tert*-butyl hydroperoxide (5 mL, 40 mmol) and stirred at rt for 1 h. The mixture was diluted with CH_2Cl_2 and washed three times with 5% $NaHCO_3$ (aq), and the aqueous layer was back-extracted with CH_2Cl_2. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column. Chromatography was performed first with CH_2Cl_2–hexane (1:1, v/v) containing 0.2% acetic acid and then with CH_2Cl_2 containing 0.2% acetic acid, applying a gradient of methanol (0–2%). The fractions containing **8a** were combined and concentrated to give **8a** (2.88 g, 94%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ –0.22; ^{1}H NMR ($CDCl_3$) δ 2.82 (4H, t, J = 6.3 Hz, CH_2CN), 4.43–4.55 (4H, m, $POCH_2$), 4.68–4.92 (3H, m, 4′-H, 5′-H, and 5″-H), 6.23 (1H, dd, $J_{3',4'}$ = 5.0 Hz, $J_{3',2'}$ = 5.3 Hz, 3′-H), 6.39 (1H, dd, $J_{2',3'}$ = 5.3 Hz, $J_{2',1'}$ = 5.0 Hz, 2′-H) 6.48 (1H, d, $J_{1',2'}$ = 5.0 Hz, 1′-H), 7.43–7.58 (9H, m, Bz), 7.91–8.07 (6H, m, Bz), 8.42 (1H, s, 2-H), 8.49 (1H, s, 8-H); ^{13}C NMR ($CDCl_3$) δ 19.6 (d, J_{POCC} = 9.8 Hz, CH_2CN), 62.4 (d, J_{POC} = 2.5 Hz, $POCH_2$), 63.5 (5′-C), 71.3 (3′-C), 73.9 (2′-C), 80.6 (4′-C), 87.0 (1′-C), 116.6 (5-C), 121.9 (d, J_{POCCC} = 12.2 Hz, CN), 128.3, 128.4, 128.5, 128.6 (3,5-C of Bz), 129.2, 129.4, 129.6, 129.7 (2,6-C of Bz), 133.5, 133.7, 133.7 (4-C of Bz), 142.1 (8-C), 150.7 (4-C), 151.4 (2-C), 152.3 (6-C), 165.1, 165.2, 166.1 (C=O of Bz). Anal. Calcd for $C_{37}H_{32}N_7O_{10}P\cdot{}^1/_2H_2O$: C, 57.37; H, 4.29; N, 12.64. Found: C, 57.29; H, 4.59; N, 12.59.

Disodium Adenosine 6-*N*-Phosphoramidate (5). 2′,3′,5′-Tri-*O*-benzoyladenosine 6-*N*-[*O,O*-bis(2-cyanoethyl)phosphoramidate] (**8a**) (0.574 g, 0.75 mmol) was treated with concentrated NH_3–pyridine (9:1, v/v, 20 mL) at rt for 12 h. The mixture was evaporated under reduced pressure, and the residue was dissolved in water. The aqueous solution was washed three times with ether and concentrated to a small volume. The residue was applied to a column of cation exchange resin (Dowex 50Wx8, Na^+ form, 300 × 15 mm) and eluted with water. The eluate was concentrated to a small volume and applied to a column of Sephadex G-10 (300 × 15 mm) eluted with water. The fractions containing **5** were combined and lyophilized to give **5** with a small amount of impurity. The crude **5** was subjected to a C18 reversed-phase column (80 × 20 mm), and elution was performed with water, applying a gradient of MeCN (0–10%). The fractions containing **5** were combined and lyophilized to give **5** (0.268 g, 91%) as a white powder: ^{31}P NMR (D_2O) δ –2.73; IR (KBr), ν 560, 625, 705, 800, 840, 875, 900, 980, 1035, 1094, 1140, 1165, 1260, 1310, 1415, 1440, 1485, 1545, 1595, and 1615 cm^{-1}; HRMS (FAB$^-$) 392.0348, calcd for $C_{10}H_{12}N_5O_7PNa_2$ 392.0291. Anal. Calcd for $C_{10}H_{12}N_5O_7PNa_2\cdot{}^1/_2H_2O$: C, 28.72; H, 3.62; N, 16.74. Found: C, 28.81; H, 3.89; N, 10.88.

2′,3′,5′-Tri-*O*-benzoyladenosine 6-*N*-[*O,O*-Bis(2-cyanoethyl)phosphorothioamidate] (9a). A procedure similar to that described in the case of **8a**, using *N,N,N′,N′*-tetraethylthiuram disulfide (4.5 equiv in MeCN at rt for 2 h) in place of *tert*-butyl hydroperoxide, gave **9a** (0.381 g, 52%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 63.7; ^{1}H NMR ($CDCl_3$) δ 2.81 (4H, t, J = 6.3Hz, CH_2CN), 4.41–4.54 (4H, m, $POCH_2$), 4.71 (1H, dd, $J_{5'',5'}$ = 11.9 Hz, $J_{5'',4'}$ = 4.3 Hz, 5″-H), 4.84 (1H, ddd, $J_{4',3'}$ = 5.0 Hz, $J_{4',5'}$ = 3.3 Hz, $J_{4',5''}$ = 4.3 Hz, 4′-H), 4.91 (1H, dd, $J_{5',5''}$ = 12.9 Hz, $J_{5',4'}$ = 3.3 Hz, 5′-H), 6.23 (1H, dd, $J_{3',4'}$ = 5.0 Hz, $J_{3',2'}$ = 5.3 Hz, 3′-H), 6.38 (1H, dd, $J_{2',3'}$ = 5.3 Hz, $J_{2',1'}$ = 5.0 Hz, 2′-H) 6.43 (1H, d, $J_{1',2'}$ = 5.0 Hz, 1′-H), 7.16–7.62 (11H, m, ArH), 7.91–8.11 (7H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 19.4 (d, J_{POCC} = 8.5 Hz, CH_2CN), 62.4 (d, J_{POC} = 3.7 Hz, $POCH_2$), 63.6 (5′-C), 71.4 (3′-C), 73.9 (2′-C), 80.8 (4′-C), 87.2 (1′-C), 116.5 (5-C), 128.3, 128.6, 128.6 (3,5-C of Bz), 129.3, 129.7, 129.8 (2,6-C of Bz), 133.4, 133.7, 133.8 (4-C of Bz), 141.8 (8-C), 150.7 (2-C), 165.1, 165.3, 166.1 (C=O of Bz). Anal. Calcd for $C_{37}H_{32}N_7O_9PS$: C, 56.85; H, 4.13; N, 12.54; S, 4.10. Found: C, 57.11; H, 4.41; N, 12.86; S, 5.12.

2′,3′,5′-Tri-*O*-acetyladenosine 6-*N*-[*O,O*-Diethyl phosphoramidate] (11b). Method A. To a mixture of 1,2,4-triazole (0.257 g, 3.7 mmol, dried by repeated coevaporation with dry pyridine and MeCN) and triethylamine (0.5 mL, 3.55 mmol) in dry MeCN (8 mL) at 0 °C was added phosphorus oxychloride (0.077 mL, 0.83 mmol). The reaction mixture was stirred at rt for 30 min. The supernatant was withdrawn by a syringe and added to a suspension of 2′,3′,5′-tri-*O*-acetyladenosine (**1b**) (0.163 g, 0.413 mmol, dried by repeated coevaporation with dry pyridine) in dry pyridine (4 mL). After being stirred for 1.5 h, the mixture was evaporated under reduced pressure, and dry ethanol (8 mL) was added to the residue. The mixture was stirred at rt for 1 h and concentrated to dryness. The residue was dissolved with $CHCl_3$ and washed three times with 5% $NaHCO_3$ (aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined and dried over Na_2SO_4, filtered, and concentrated to dryness. Silica gel column chromatography with CH_2Cl_2 containing 0.2% acetic acid, applying a gradient of methanol (0–1.5%), gave **11b** (0.93 g, 43%) as a hygroscopic foam.

Method B. 2′,3′,5′-Tri-*O*-acetyladenosine (**1b**) (0.399 g, 1.01 mmol) was dried by repeated coevaporation with dry pyridine and finally suspended in dry pyridine (10 mL). To the suspension was added diethyl phosphorochloridite (0.294 mL, 2.02 mmol) and triethylamine (0.283 mL, 2.02 mmol). After being stirred at rt for 1 h, the mixture was treated with *tert*-butyl hydroperoxide (4.1 mL, 12.1 mmol) and stirred at rt for 1 h. The mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$ (aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column and eluted with CH_2Cl_2 containing 0.2% acetic acid, applying a gradient of methanol (0–1.5%). The fractions containing **11b** were combined and concentrated to give **11b** (0.491 g, 91%) as a hygroscopic foam: ^{31}P NMR ($CDCl_3$) δ –0.75; ^{1}H NMR ($CDCl_3$) δ 1.36 (6H, 2t, J = 7.0 Hz, CH_3 of Et), 2.09, 2.11, 2.16 (9H, 3s, Ac), 4.04–4.43 (7H, m, $POCH_2$, 5′-H, 5″-H, and 4′-H), 5.70 (1H, t, $J_{3',2'}$ = 5.4 Hz, 3′-H), 6.01 (1H, t, $J_{2',3'}$ = 5.4 Hz, 2′-H) 6.25 (1H, d, $J_{1',2'}$ = 5.1 Hz, 1′-H), 8.46 (1H, s, 2-H), 8.63 (1H, s, 8-H); ^{13}C NMR ($CDCl_3$) δ 15.9 (d, J_{POCC} = 7.3 Hz, CH_3 of Et), 20.2, 20.3, 20.5 (Ac), 62.9 (5′-C), 63.7 (d, J_{POC} = 4.9 Hz, $POCH_2$), 70.4 (3′-C), 72.8 (2′-C), 80.4 (4′-C), 86.2 (1′-C), 121.5 (d, J_{PNCC} = 12.1 Hz, 5-C), 141.4 (8-C), 150.4 (4-C), 151.9 (2-C), 152.7 (6-C), 169.3, 169.5, 170.3 (C=O of Ac).

2′,3′,5′-Tris-*O*-(phenoxyacetyl)adenosine 6-*N*-[*O,O*-Diethyl phosphoramidate] (11c). 2′,3′,5′-Tris-*O*-(phenoxyacetyl)adenosine (**1c**) (0.134 g, 0.2 mmol) and 1*H*-tetrazole (0.032 g, 0.45 mmol) were dried by repeated coevaporation with dry pyridine followed by dry MeCN and dissolved in dry MeCN (2 mL). To the mixture of **1c** and 1*H*-tetrazole in dry MeCN was added diethoxy(*N,N*-diisopropylamino)phosphine (0.089 g, 0.4 mmol). After being stirred at rt for 1 h, *tert*-butyl hydroperoxide (0.25 mL, 2 mmol) was added, and the mixture was stirred at rt for 1 h. The mixture was diluted with CH_2Cl_2, washed three times with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with CH_2Cl_2. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column. Chromatography was performed with CH_2Cl_2 containing 0.2% acetic acid, applying a gradient of methanol (0–1.5%). The fractions containing **11c** were combined and concentrated to give **11c** (0.161 g,

81%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ −1.32; ^{1}H NMR ($CDCl_3$) δ 1.37 (6H, t, J = 7.3 Hz, CH_3 of Et), 4.20–4.30 (4H, m, $POCH_2$), 4.32–4.69 (9H, m, CH_2 of Pac, 4′-H, 5′-H, and 5″-H), 5.81 (1H, dd, $J_{3',4'}$ = 4.3 Hz, $J_{3',2'}$ = 5.3 Hz, 3′-H), 6.06–6.18 (2H, m, 1′-H and 2′-H), 6.78–7.03 (9H, m, 3,4,5-H of Ph), 7.19–7.33 (7H, m, 2,6-H of Ph and NH), 8.10 (1H, s, 2-H), 8.49 (1H, s, 8-H); ^{13}C NMR ($CDCl_3$) δ 16.1 (d, J_{POCC} = 7.3 Hz, CH_3 of Et), 63.3 (5′-C), 63.9 (d, J_{POC} = 6.1 Hz, $POCH_2$), 64.7, 64.8, 65.1 (CH_2 of Pac), 71.3 (3′-C), 73.2 (2′-C), 79.8 (4′-C), 86.0 (1′-C), 114.4, 114.5, 114.6 (5-C and 2,6-C of Ph), 121.9, 122.0, 122.1 (4-C of Ph), 129.5, 129.6, 129.7 (3,5-C of Ph), 141.4 (8-C), 150.4 (4-C), 151.9 (2-C), 152.8 (6-C), 157.3, 157.4 (1-C of Ph), 167.7, 168.0, 168.4 (C=O of Pac). Anal. Calcd for $C_{38}H_{40}N_5O_{13}P$: C, 56.65; H, 5.00; N, 8.69. Found: C, 56.68; H, 5.10; N, 9.00.

Adenosine 6-*N*-[*O*,*O*-Diethyl phosphoramidate] (12). 2′,3′,5′-Tris-*O*-(phenoxyacetyl)adenosine 6-*N*-[*O*,*O*-diethyl phosphoramidate] (**11c**) (0.403 g, 0.5 mmol) was treated with concentrated NH_3–pyridine–water (5:40:55, v/v/v, 50 mL) at rt for 30 min. The mixture was evaporated to dryness, and the residue was dissolved in water and washed three times with ether. The aqueous layer was concentrated to a small volume and applied to a C18 reversed-phase column (18 × 20 mm) and eluted with water, applying a gradient of MeCN (0–6%). The fractions containing **12** were combined and lyophilized to give **12** (0.127 g, 63%) as a white powder: ^{31}P NMR (D_2O) δ 1.30; IR (KBr) ν 740, 865, 975, 1025, 1160, 1225, 1325, 1355, 1440, 1455, 1580, and 1605 cm^{-1}. Anal. Calcd for $C_{14}H_{22}N_5O_7P$: C, 41.69; H, 5.50; N, 17.36. Found: C, 41.65; H, 5.55; N, 16.78.

2′,3′,5′-Tri-*O*-acetyladenosine 6-*N*-[*O*,*O*-Diethyl phosphorothioamidate] (13b). 2′,3′,5′-Tri-*O*-acetyladenosine (**1b**) (0.393 g, 1.42 mmol) was dried by repeated coevaporation with dry pyridine and suspended in dry pyridine (20 mL). To the above suspension were added diethyl phosphorochloridite (0.587 mL, 2.84 mmol) and triethylamine (0.565 mL, 2.84 mmol). After being stirred at rt for 1 h, a solution of S_8 (3.1 g, 100 mmol) in CS_2 (10 mL) was added, and the mixture was stirred at rt for 22 h. The mixture was evaporated to a small volume, and excess S_8 was filtered. The filtrate was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with $CHCl_3$. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column and eluted first with CH_2Cl_2–hexane (2:8, v/v) containing 0.5% triethylamine followed with CH_2Cl_2–hexane (4:6, v/v) containing 0.5% triethylamine. The fractions containing **13b** were combined and concentrated to give **13b** (0.403 g, 52%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 63.96; ^{1}H NMR ($CDCl_3$) δ 1.35 (6H, t, J = 7.0 Hz, CH_3 of Et), 2.08, 2.12, 2.15 (9H, 3s, Ac), 4.11–4.44 (7H, m, $POCH_2$, 4′-H, 5′-H, and 5″-H), 5.68 (1H, t, J = 5.4 Hz, 3′-H), 5.96 (1H, dd, $J_{2',3'}$ = 5.3 Hz, $J_{2',1'}$ = 5.1 Hz, 2′-H) 6.20 (1H, d, $J_{1',2'}$ = 5.1 Hz, 1′-H), 8.11 (1H, s, 2-H), 8.62 (1H, s, 8-H); ^{13}C NMR ($CDCl_3$) δ 15.5 (d, J_{POCC} = 7.3 Hz, CH_3 of Et), 20.2, 20.3, 20.6 (Ac), 62.8 (5′-C), 64.7 (d, J_{POC} = 17.1 Hz, $POCH_2$), 70.4 (3′-C), 72.9 (2′-C), 80.2 (4′-C), 86.2 (1′-C), 121.1 (5-C), 143.4 (8-C), 151.9, 152.2, 153.0 (2-C, 4-C, and 6-C), 169.1, 169.3, 170.1 (C=O of Ac).

2′,3′,5′-Tri-*O*-benzoylcytidine 4-*N*-[*O*,*O*-Bis(2-cyanoethyl)phosphoramidate] (16). 2′,3′,5′-Tri-*O*-benzoylcytidine (**14a**) (0.556 g, 1 mmol) and 1*H*-tetrazole (0.105 g, 1.5 mmol) were dried by repeated coevaporation with dry pyridine followed by dry MeCN and dissolved in dry MeCN (10 mL). To the mixture of **14a** and 1*H*-tetrazole in dry MeCN was added bis(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.407 g, 1.5 mmol). After being stirred at rt for 2 h, *tert*-butyl hydroperoxide (1.25 mL, 10 mmol) was added, and the mixture was stirred at rt for 1 h. The mixture was diluted with CH_2Cl_2, washed three times with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with CH_2Cl_2. The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column. Chromatography was performed first with CH_2Cl_2–hexane (1:1, v/v) containing 0.2% acetic acid followed with CH_2Cl_2 containing 0.2% acetic acid, applying a gradient of methanol (0–1.5%). The fractions containing **16** were combined and concentrated to give **16** (0.441 g, 61%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 6.03; ^{1}H NMR ($CDCl_3$) δ 2.76 (4H, t, J = 6.3 Hz, CH_2CN), 4.21–4.29 (4H, m, $POCH_2$), 4.67–4.85 (3H, m, 4′-H, 5′-H, and 5″-H), 5.73 (1H, dd, $J_{2',3'}$ = 5.9 Hz, $J_{2',1'}$ = 5.3 Hz, 2′-H), 5.86–5.91 (2H, m, 3′-H and 5-H), 6.29 (1H, d, $J_{1',2'}$ = 5.3 Hz, 1′-H), 7.34–7.63 (10H, m, Bz, 6-H and NH), 7.92–8.11 (6H, m, Bz); ^{13}C NMR ($CDCl_3$) δ 19.7 (d, J_{POCC} = 7.4 Hz, CH_2CN), 61.3 (d, J_{POC} = 6.1 Hz, $POCH_2$), 63.6 (5′-C), 71.1 (3′-C), 73.8 (2′-C), 80.6 (4′-C), 88.5 (1′-C), 103.3 (d, J_{POCCC} = 20.8 Hz, CN), 116.6 (5-C), 128.3, 128.6, 128.8, 129.1, 129.6, 129.8, 129.9, 133.7, 133.8, 133.9 (Bz), 139.9 (6-C), 148.0 (2-C), 159.7 (4-C), 165.3, 166.0 (C=O of Bz). Anal. Calcd for $C_{36}H_{32}N_5O_{11}P\cdot{}^1/_2H_2O$: C, 57.60; H, 4.43; N, 9.33. Found: C, 57.33; H, 4.90; N, 9.83.

Disodium Cytidine 4-*N*-Phosphoramidate (17). 2′,3′,5′-Tri-*O*-benzoylcytidine 4-*N*-[*O*,*O*-bis(2-cyanoethyl) phosphoramidate] (**16**) (0.794 g, 1.09 mmol) was treated with concentrated NH_3–pyridine (9:1, v/v, 40 mL) at rt for 12 h. Ammonia and pyridine were removed by evaporation, and the residue was dissolved in water and washed three times with ether. The aqueous layer was concentrated to a small volume and applied to a column of cation exchange resin (Dowex 50Wx8, Na^+ form, 300 × 15 mm) and eluted with water. The eluate was concentrated to a small volume and applied to a column of Sephadex G-10 (300 × 15 mm) and eluted with water. The fractions containing **3** were combined and lyophilized to give **17** with a small impurity. The crude **17** was purified on a C18 reversed-phase column (80 × 20 mm) and eluted with water, applying a gradient of MeCN (0–15%). The fractions containing **3** were combined and lyophilized to give **17** (0.307 g, 76%) as a white powder: ^{31}P NMR (D_2O) δ −3.15; IR (KBr) ν 545, 625, 780, 840, 970, 1065, 1120, 1435, 1475, 1535, 1620, and 1630 cm^{-1}; HRMS (FAB^-) 366.0113, calcd for $C_9H_{11}N_3O_8PNNa_2$ 366.1550.

Cytidine 4-*N*-[*O*,*O*-Diethyl phosphoramidate] (20). Cytidine (0.486 g, 2 mmol) was dried by repeated coevaporation with dry pyridine and suspended in dry pyridine–MeCN (1:1, v/v, 20 mL). To the above suspension was added hexamethyldisilazane (2.1 mL, 10 mmol), and the mixture was refluxed for 2 h. The reaction mixture was cooled to rt and concentrated to give a colorless foam. The resulting foam was dissolved in dry MeCN (20 mL), and diethoxy(*N*,*N*-diisopropylamino)phosphine (0.893 g, 4 mmol) and 1*H*-tetrazole (0.350 g, 10 mmol) were added. After being stirred at rt for 2 h, *tert*-butyl hydroperoxide (2.5 mL, 20 mmol) was added, and the mixture was stirred at rt for 30 min. The reaction was quenched with water, and the mixture was concentrated to dryness. The residue was dissolved in water and applied to a C18 reversed-phase column (80 × 20 mm) and eluted with water, applying a gradient of MeCN (0–6%). The fractions containing **20** were combined and lyophilized to give **20** (0.572 g, 75%) as a white powder: ^{31}P NMR (D_2O) δ 2.95; IR (KBr) ν 505, 945, 1010, 1190, 1445, 1540, and 1600 cm^{-1}.

2′,3′,5′-Tri-*O*-acetylguanosine 2-*N*-[*O*,*O*-Bis(2-cyanoethyl) phosphoramidate] (24). 2′,3′,5′-Tri-*O*-acetylguanosine (**21**) (0.819 g, 2.0 mmol) was dried by repeated coevaporation with dry pyridine and dissolved in dry pyridine (20 mL). Trimethylsilyl chloride (1.02 mL, 8.0 mmol) was added to the above mixture and stirred at rt for 30 min. To the mixture was added bis(2-cyanoethoxy)(*N*,*N*-diisopropylamino)phosphine (0.814 g, 3.0 mmol). After being stirred at rt for 2 h, *tert*-butyl hydroperoxide (2.5 mL, 20 mmol) was added, and the mixture was stirred at rt for 30 min. The reaction was quenched by the addition of water, and the mixture was diluted with CH_2Cl_2–pyridine (2:1, v/v, 40 mL). The organic layer was washed three times with 5% $NaHCO_3$(aq), and the aqueous layer was back-extracted with CH_2Cl_2–pyridine (2:1, v/v). The organic layer and washings were combined, dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was applied to a silica gel column. Chromatography was performed with CH_2Cl_2, applying a gradient of methanol (0–4%). The fractions containing **24** were combined and concentrated to a colorless foam. The product was recrystallized from MeOH (20 mL) to give **24** (0.304 g, 26%) as a crystal: mp 166–168 °C; ^{31}P NMR ($CDCl_3$:CD_3OD = 2:1, v/v) δ −1.05; ^{1}H NMR ($CDCl_3$:CD_3OD = 2:1) δ 2.11, 2.14, 2.16 (9H, 3s, Ac), 2.91 (4H, t, J = 3.6 Hz, CH_2CN), 4.36–4.49 (7H, m, $POCH_2$, 4′-H, 5′-H and 5″-H), 5.52 (1H, t, $J_{3',4'}$ = $J_{3',2'}$ = 5.6 Hz, 3′-H), 5.99 (1H, dd, $J_{2',3'}$ = 5.6 Hz, $J_{2',1'}$ = 4.6 Hz, 2′-H) 6.07 (1H, d, $J_{1',2'}$ = 4.6 Hz, 1′-H), 7.93 (1H, s, 8-H). Anal. Calcd for $C_{22}H_{27}N_7O_{11}P$: C, 44.30; H, 4.56; N, 16.43. Found: C, 44.03; H, 4.64; N, 16.51.

Disodium Guanosine 2-*N*-Phosphoramidate (25). 2′,3′,5′-Tri-*O*-acetyladenosine 2-*N*-[*O*,*O*-bis(2-cyanoethyl) phosphoramidate] (**24**) (0.119 g, 0.2 mmol) was treated with concentrated NH_3–pyridine (4:1, v/v, 20 mL) at rt for 2 d. Ammonia and pyridine were removed by evaporation, and the residue was dissolved in water and washed three times with ether. The aqueous layer was concentrated to a small volume and applied to a column of cation exchange resin (Dowex 50Wx8, Na^+ form, 300 × 15 mm) and eluted with water. The eluate was concentrated to a small volume and applied to a column of Sephadex G-10 (300 × 15 mm) and eluted with water. The fractions containing **25** were combined and lyophilized to give **25** (0.078 g, 99%) as a white powder: ^{31}P NMR

(D_2O) δ −1.73; IR (KBr) ν 535, 630, 965, 1070, 1090, 1320, 1555, 1580, and 1645 cm^{-1}; HRMS (FAB$^-$) 362.0473, calcd for $C_{10}H_{13}N_5O_8P$ 362.2152.

2′,3′,5′-Tri-*O*-acetylguanosine 2-*N*-[*O,O*-Diethyl phosphoramidate] (27). By the same procedure as described for **24**, diethoxy(*N,N*-diisopropyl)aminophosphine was used in place of bis(2-cyanoethoxy)-(*N,N*-diisopropylamino)phosphine to give **27** (0.123 g, 45%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ −0.43; ^1H NMR ($CDCl_3$:CD_3OD (2:1, v/v) δ 1.37 (6H, t, J = 7.3 Hz, CH_3 of Et), 2.09, 2.12, 2.13 (9H, 3s, Ac), 4.17–4.29 (4H, m, $POCH_2$), 4.39–4.54 (3H, m, 4′-H, 5′-H and 5″-H), 5.63 (1H, dd, $J_{3',4'}$ = 4.3 Hz, $J_{3',2'}$ = 4.6 Hz, 3′-H), 5.96 (2H, dd, $J_{2',3'}$ = 4.6 Hz, $J_{2',1'}$ = 5.0 Hz, 2′-H and 1′-H), 7.69 (1H, s, 8-H), 8.62 (1H, bs, NH). Anal. Calcd for $C_{20}H_{28}N_5O_{11}P\cdot{}^1/_2H_2O$: C, 43.33; H, 5.27; N, 12.63. Found: C, 43.27; H, 5.60; N, 12.99.

Guanosine 2-*N*-[*O,O*-Diethyl phosphoramidate] (28). 2′,3′,5′-Tri-O-acetylguanosine 2-*N*-[*O,O*-diethyl phosphoramidate] (**27**) (0.164 g, 0.3 mmol) was treated with 0.5 M NaOH–pyridine (1:1, v/v, 20 mL) at rt for 2 h. Dowex 50Wx8 (H^+ form, 300 × 15 mm) was added to neutralize the mixture, and the resin was filtered off. The filtrate was concentrated to a small volume and applied on a column of reversed-phase C18 (80 × 20 mm). Chromatography was performed with a gradient of 0–15% MeOH in water. The fractions containing **28** were combined and lyophilized to give **28** (0.108 g, 80%) as a white powder: ^{31}P NMR (D_2O) δ 0.57; IR (KBr) ν 535, 905, 960, 1020, 1050, 1160, 1235, 1280, 1360, 1445, 1550, 1575, 1600, and, 1685 cm^{-1}; Anal. Calcd for $C_{14}H_{22}N_5O_8P\cdot H_2O$: C, 38.45; H, 5.53; N, 16.01. Found: C, 38.81; H, 5.30; N, 15.93.

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Supplementary Material Available: Figures showing the ^1H, ^{13}C, and ^{31}P NMR spectra of **3a**, **5**, **6**, **11b**, **12**, **13b**, **17**, **20**, **25**, and **28** (30 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.