O-Selective Phosphorylation of Nucleosides without N-Protection[1]

Mamoru Uchiyama,[†] Yoshio Aso,[‡] Ryoji Noyori,*[,†] and Yoshihiro Hayakawa*[,§]

Department of Chemistry, Faculty of Science, Chemical Instrument Center, and Division of Informatics for Sciences, Graduate School of Human Informatics, Nagoya University, Chikusa, Nagoya 464-01, Japan

Received July 20, 1992

A facile chemoselective O-phosphorylation of N-unprotected nucleosides has been achieved via metal alkoxide formation. Sequential treatment of N-unprotected nucleosides with an equimolar amount of a metallo-organic base such as an alkyllithium, potassium *tert*-butoxide, *tert*-butylmagnesium chloride, $LiAl[N(CH_3)_2]_4$, $Al[N(CH_3)_2]_3$, $(i\text{-}C_4H_9)_2AlH$, etc., and a phosphorochloridate or *p*-nitrophenyl phosphate results in rapid O-phosphorylation to give the corresponding nucleotides in high yield. The method using magnesium alkoxides is among the best, considering its chemoselectivity, generality, and operational simplicity. The origin of the observed chemoselectivity is discussed.

Introduction

Solution-phase chemical synthesis of nucleic acids[2] has significant potential in drug synthesis and is particularly appropriate for large-scale preparation of short-sequence oligonucleotides.[3-7] Low-cost reagents and operational simplicity allowing large-scale, reproducible syntheses are important practical considerations. Most existing procedures, however, do not generally satisfy these conditions. An essential process in nucleotide synthesis is phosphorylation of a hydroxyl function of a nucleoside. Since the hydroxyl groups, particularly the 2′- and 3′-hydroxyls in nucleosides do not react easily with ordinary phosphoryl chlorides,[8] the condensation is conventionally achieved by using strongly electrophilic phosphorylating agents formed from a phosphoric acid derivative and a sterically congested arenesulfonyl chloride such as 2,4,6-triisopropylbenzenesulfonyl chloride or the corresponding azolides.[2,9] The activating agents are expensive, and the procedures are not operationally simple. Furthermore, under ordinary conditions, the phosphorylation of nucleosides, such as adenosine or cytidine, which have hydroxyl and amino groups in the same molecule, takes place competitively at both the amino and hydroxyl functions,[10] and hence the amino groups must be protected in order to achieve the selective O-phosphorylation.[8] The deprotection very often brings about undesired side reactions, particularly cleavage of the phosphate linkage, resulting in serious loss of the product.

Direct O-phosphorylation of nucleosides without N-protection is obviously ideal. Our approach is based on a different, very simple concept, namely *functional group activation* rather than protection. When a nucleoside is treated with 1 equiv of a strong metallo-organic base, an equilibrium mixture of the metal alkoxide 1 and amide 3 is formed, as illustrated in Scheme I. In principle, predominance of O- or N-phosphorylation depends on the equilibrium concentration of 1 and 3 and their relative reactivities. Therefore, in going from the reaction of the neutral alcohols or amines to the strong base-promoted reaction, a change in the chemoselectivity profile can be expected, hopefully in such a way as to selectively afford the phosphate 2.[11] In addition, the enhanced nucleophilicity of the oxygen function may allow the use of readily available phosphorylating agents. We here describe a facile O-selective phosphorylation of N-unblocked nucleosides via hydroxyl activation.

[†] Department of Chemistry, Faculty of Science.
[‡] Chemical Instrument Center.
[§] Division of Informatics for Sciences, Graduate School of Human Informatics.

(1) For preliminary reports of this work, see: (a) Hayakawa, Y.; Aso, Y.; Uchiyama, M.; Noyori, R. *Tetrahedron Lett.* **1983**, *24*, 1165. (b) Hayakawa, Y.; Aso, Y.; Uchiyama, M.; Noyori, R. *Ibid.* **1983**, *24*, 5641.

(2) For comprehensive reviews on oligonucleotide synthesis: (a) Hata, T.; Matsuzaki, J. *J. Synth. Org. Chem., Jpn.* **1984**, *42*, 429. (b) Crockett, G. C. *Aldrichimica Acta* **1983**, *16* (3), 47. (c) Narang, S. A. *Tetrahedron* **1983**, *39*, 3. (d) Ikehara, M.; Ohtsuka, E.; Markham, A. F. *Adv. Carbohydr. Chem. Biochem.* **1979**, *36*, 135. (e) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143. (f) Amarnath, V.; Broom, A. D. *Chem. Rev.* **1977**, *77*, 183. (g) Zhdanov, R. I.; Zhenodarova, S. M. *Synthesis* **1975**, 222. (h) Kössel, H.; Seliger, H. *Fortschr. Chem. Org. Naturst.* **1975**, *32*, 297. (i) Khorana, H. G. *Pure Appl. Chem.* **1968**, *17*, 349.

(3) 2′,5′-Linked oligoadenylates (2-5As): (a) Kerr, I. M.; Brown, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 256. (b) *The 2-5A System: Molecular and Clinical Aspects of the Interferon-Regulated Pathway*; Williams, B. R. G., Silverman, R. H., Eds.; Alan R. Liss: New York, 1985. Syntheses: (c) Hayakawa, Y.; Uchiyama, M.; Norbori, T.; Noyori, R. *Tetrahedron Lett.* **1985**, *26*, 761. (d) Noyori, R.; Uchiyama, M.; Norbori, T.; Hirose, M.; Hayakawa, Y. *Aust. J. Chem.* **1992**, *45*, 205 and references cited therein.

(4) Branch-type RNAs: (a) Wallace, J. C.; Edmonds, M. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 950. Syntheses: (b) Hayakawa, Y. *J. Synth. Org. Chem., Jpn.* **1988**, *46*, 753 and references cited therein.

(5) Nucleotide phospholipids: (a) Ryu, E. K.; Ross, R. J.; Matsushita, T.; MacRoss, M.; Hong, C. I.; West, C. R. *J. Med. Chem.* **1982**, *25*, 1322. Syntheses: (b) Ramirez, F.; Mandal, S. B.; Marecek, J. F. *Synthesis* **1982**, 402. (c) Ramirez, F.; Mandal, S. B.; Maracek, J. F. *J. Am. Chem. Soc.* **1982**, *104*, 5483. (d) Murthy, P. P. N.; Agranoff, B. W. *Biochim. Biophys. Acta* **1982**, *712*, 473.

(6) Nucleotide steroids: Tsoupras, G.; Hetru, C.; Luu, B.; Constantin, E.; Lagueux, M.; Hoffmann, J. *Tetrahedron* **1983**, *39*, 1789.

(7) CMP sialic acid: (a) Sabesan, S.; Paulson, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 2068. (b) Palcic, M. M.; Venot, A. P.; Ratcliffe, R. M.; Hindsgaul, O. *Carbohydr. Res.* **1989**, *190*, 1. Enzymatic syntheses: (c) Simon, E. S.; Bednarski, M. D.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 7159 and references cited therein.

(8) Condensation of phosphorus oxychloride and an unprotected nucleoside in a trialkyl phosphate or in acetonitrile or pyridine containing a small amount of water gives selectively, after hydrolysis, the nucleoside 5′-monophosphate in high yield. However, the 2′- or 3′-hydroxyl group is little phosphorylated. (a) Yoshikawa, M.; Kato, T. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2849. (b) Yoshikawa, M.; Kato, T.; Takenishi, T. *Ibid.* **1969**, *42*, 3505. (c) Sowa, T.; Ouchi, S. *Ibid.* **1975**, *48*, 2084.

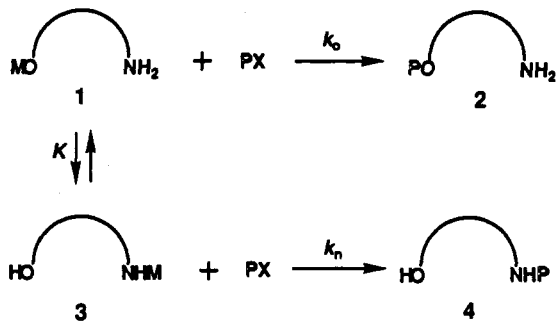
(9) (a) Zarytova, V. F.; Knorre, D. G. *Nucleic Acids Res.* **1984**, *12*, 2091. (b) Chandrasegaran, S.; Murakami, A.; Kan, L. *J. Org. Chem.* **1984**, *49*, 4951.

(10) Tener, G. M. *J. Am. Chem. Soc.* **1961**, *83*, 159.

(11) Existing methods for phosphorylation of nucleosides via hydroxyl activation. With sodium metal as an activator: Škarić, V.; Hohnjec, M.; Skaric, D. *Croat. Chem. Acta* **1977**, *49*, 851. With potassium *tert*-butoxide: Borden, R. K.; Smith, M. *J. Org. Chem.* **1966**, *31*, 3247. With dibutylstanoxane: Wagner, D.; Verheyden, J. P. H.; Moffatt, G. *Ibid.* **1974**, *39*, 24. Application of our method using *tert*-butylmagnesium chloride: Jaworska, M.; Lesnikowski, Z. J.; Stec, W. J. *Nucleic Acids Res., Symp. Ser.* **1989**, *21*, 99 and references cited therein.

0022-3263/93/1958-0373$04.00/0

Scheme I



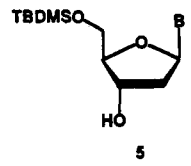
PX = phosphorylating agent
M = metallic species

Results and Discussion

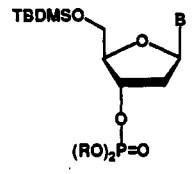
Phosphorylation of Nucleosides. First, we screened organometallic reagents which act as strong bases to effect phosphorylation of N-unprotected nucleosides **5**, **8**, and **10** using phosphorochloridates or *p*-nitrophenyl phosphates. The results are listed in Table I. When the adenosine nucleoside **5a** was treated with $(C_2H_5O)_2POCl$ in pyridine, a mixture of the N^6-phosphorylated product (26%) and the 3′-phosphate **7a** (14%) was obtained. In contrast, in the presence of *tert*-butylmagnesium chloride the reaction of **5a** and phosphorochloridate or *p*-nitrophenyl phosphate in THF gave the phosphate **7a** as a sole product (entries 10 and 11). Thus, addition of the Grignard reagent strongly effects the product distribution and results in exclusive O-phosphorylation of the N-unprotected nucleoside. *tert*-Butylmagnesium chloride was found to be one of the best, general reagents for the O-selective phosphorylation. The reaction can be performed with a variety of N-unblocked nucleosides on any large scale and with high selectivity. The nucleoside/Grignard reagent stoichiometry is also important to obtain both a fast reaction rate and a high yield of the desired product. With adenosines and cytidines, the O-selective condensation was simply accomplished at ambient temperature by using 1 equiv of the Grignard reagent and 1.2–1.4 equiv of the phosphorylating agent. When an excess of the activator was used in these cases, the N,O-diphosphorylated compound was produced in a considerable amount. The reaction of guanosine, thymidine, and uridine derivatives proceeds rather slowly with 1 equiv of the Grignard reagent, although the product yields are acceptable (>88%). Two equivalents of the Grignard reagent completes the reaction in a short period. High-quality Grignard reagent is also crucial for obtaining high product yield. The purity of *tert*-butylmagnesium chloride employed here was higher than 90% (organomagnesium/total base).

Activation of a nucleoside, NuOH, as the tetravalent lithium aluminate, $Li[Al(OAr)_3ONu]$ (empirical formula), is also applicable for O-selective phosphorylation, although the reactivity is rather low.[12] The trivalent aluminum alkoxides, $Al(OAr)_2ONu$ and $(i\text{-}C_4H_9)_2Al(ONu)$, showed similar reactivity. In the aluminum alkoxides containing ArO moieties, the structure of Ar strongly affected efficiency of the phosphorylation. Use of $p\text{-}NO_2C_6H_4OH$ or $o\text{-}ClC_6H_4OH$ as ArOH generally gave satisfactory results.

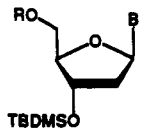
(12) The reaction of the aluminate is slow and requires more forcing conditions than that of the lithium alkoxide.



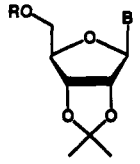
5



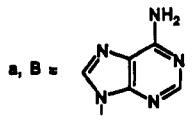
6, R = *o*-ClC_6H_4
7, R = C_2H_5

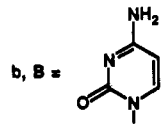


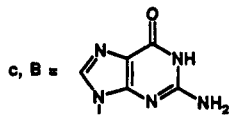
8, R = H
9, R = $(o\text{-}ClC_6H_4O)_2PO$

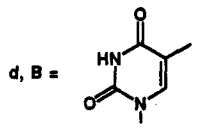


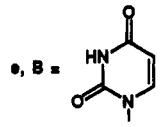
10, R = H
11, R = $(o\text{-}ClC_6H_4O)_2PO$
12, R = $(C_2H_5O)_2PO$











TBDMS = $t\text{-}C_4H_9(CH_3)_2Si$

A drastic decrease in product yield and reactivity was observed in the reaction using C_6H_5OH, $o\text{-}CH_3C_6H_4OH$, $p\text{-}CH_3C_6H_4OH$, $2,6\text{-}(t\text{-}C_4H_9)_2C_6H_3OH$, $o\text{-}NO_2C_6H_4OH$, $2,4\text{-}(NO_2)_2C_6H_3OH$, $p\text{-}ClC_6H_4OH$, or 2-naphthol. As a base promoter, various alkyllithium reagents can also be employed for the phosphorylation of adenosines at −78 °C, giving the desired N-free phosphates in excellent yields. Unfortunately, however, this method is not general. For instance, reaction of N-unprotected cytosinyl nucleoside and phosphorochloridate or *p*-nitrophenyl phosphate in the presence of *tert*-butyllithium led to a mixture of the desired O-phosphorylated product, N^4-phosphorylated compound, and N^4,O-diphosphorylated derivative (entries 15 and 16).[1] The guaninyl nucleoside gave no O-phosphorylated product mainly due to the insolubility of the lithiated nucleosides. Potassium *tert*-butoxide showed similar reactivity and was usable only for the phosphorylation of an adeninyl nucleoside (entry 9).

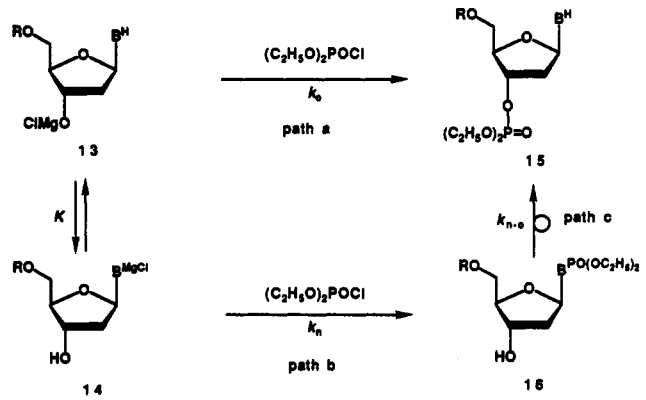
Mechanism of the Chemoselective Phosphorylation. The observed selectivity is illustrated by the pathway outlined in Scheme II. In order to elucidate the origin of the chemoselectivity and preferential pathway observed in the phosphorylation of nucleosides via hydroxyl activation, we examined the relative stabilities of magnesium alkoxides and amides as well as their reactivities. Reaction of equimolar amounts of a nucleoside and *tert*-butylmagnesium chloride eliminates isobutane to generate the

Table I. Phosphorylation of N-Unprotected Nucleosides via Hydroxyl Activation[a]

			$(RO)_2POX$		conditions			
entry	nucleoside	metalating agent (equiv)	R	X	temp, °C	time, h	nucleotide	% yield[b]
1	**5a**	CH_3Li (1)	*o*-ClC_6H_4	Cl	−78	1	**6a**	90
2	**5a**	*n*-C_4H_9Li (1)	*o*-ClC_6H_4	Cl	−78	1	**6a**	93
3	**5a**	*t*-C_4H_9Li (1)	*o*-ClC_6H_4	Cl	−78	1.25	**6a**	95, 91[c]
4	**5a**	MesLi[d] (1)	*o*-ClC_6H_4	Cl	−78	0.25	**6a**	97, 92[c]
5	**5a**	MesLi (1)	*o*-ClC_6H_4	CF_3CO_2	−78	3	**6a**	65
6	**5a**	MesLi (1)	C_2H_5	*p*-$NO_2C_6H_4O$	−78	3	**7a**	84
7	**5a**	2,6-DMPLi[e] (1)	*o*-ClC_6H_4	Cl	−78	2	**6a**	88
8	**5a**	NaH (1)	*o*-ClC_6H_4	Cl	−78	1	**6a**	72
9	**5a**	*t*-C_4H_9OK (1)	C_2H_5	Cl	−78	1	**7a**	90
10	**5a**	*t*-C_4H_9MgCl (1)	C_2H_5	Cl	24	1	**7a**	94, 91[c]
11	**5a**	*t*-C_4H_9MgCl (1)	C_2H_5	*p*-$NO_2C_6H_4O$	24	1	**7a**	93[c]
12	**5a**	$LiAl[N(CH_3)_2]_4$[f] (1)	*o*-ClC_6H_4	Cl	0	6	**6a**	82
13	**5a**	$Al[N(CH_3)_2]_3$[g] (1)	*o*-ClC_6H_4	Cl	20	4	**6a**	67
14	**5a**	DIBAL-H[h] (1)	C_2H_5	Cl	25	2	**7a**	88
15	**5b**	*t*-C_4H_9Li (1)	*o*-ClC_6H_4	Cl	−50	4	**6b**	27[i]
16	**5b**	*t*-C_4H_9Li (1)	*o*-ClC_6H_4	*p*-$NO_2C_6H_4O$	−50	4	**6b**	9[j]
17	**5b**	*t*-C_4H_9MgCl (1)	C_2H_5	Cl	25	0.5	**7b**	95, 94[k]
18	**5b**	$LiAl[N(CH_3)_2]_4$[f] (1)	C_2H_5	Cl	0	6	**7b**	95, 86[c]
19	**5b**	$LiAl[N(CH_3)_2]_4$[f] (1)	*o*-ClC_6H_4	Cl	20	6	**6b**	73
20	**5b**	$LiAl[N(CH_3)_2]_4$[f] (1)	*o*-ClC_6H_4	*p*-$NO_2C_6H_4O$	20	3	**6b**	83[c]
21	**5b**	$Al[N(CH_3)_2]_3$[g] (1)	*o*-ClC_6H_4	Cl	27	14	**6b**	62
22	**5c**	*t*-C_4H_9MgCl (1)	C_2H_5	Cl[l]	15	6	**7c**	88
23	**5c**	*t*-C_4H_9MgCl (2)	C_2H_5	Cl[l]	25	0.5	**7c**	94, 89[c]
24	**5c**	$LiAl[N(CH_3)_2]_4$[f] (1)	*o*-ClC_6H_4	Cl	20	11	**6c**	69
25	**5d**	*t*-C_4H_9Li (2)	*o*-ClC_6H_4	Cl	−50	1.5	**6d**	81[c]
26	**5d**	*t*-C_4H_9MgCl (1)	C_2H_5	Cl[l]	15	6	**7d**	92
27	**5d**	*t*-C_4H_9MgCl (2)	C_2H_5	Cl[l]	25	0.5	**7d**	98, 91[c]
28	**5d**	$LiAl[N(CH_3)_2]_4$[f] (1)	*o*-ClC_6H_4	Cl	0	10	**6d**	88
29	**5d**	DIBAL-H (1)	C_2H_5	Cl	25	3	**7d**	70
30	**8a**	*n*-C_4H_9Li (1)	*o*-ClC_6H_4	Cl	−78	3	**9a**	85
31	**8a**	*t*-C_4H_9Li (1)	*o*-ClC_6H_4	Cl	−78	0.5	**9a**	92
32	**8a**	*t*-C_4H_9Li (1)	*o*-ClC_6H_4	*p*-$NO_2C_6H_4O$	−78	1.5	**9a**	94
33	**8d**	*t*-C_4H_9Li (2)	*o*-ClC_6H_4	Cl	−50	1	**9d**	85[c]
34	**10a**	*t*-C_4H_9Li (1)	*o*-ClC_6H_4	Cl	−78	0.5	**11a**	94, 88[c]
35	**10a**	*t*-C_4H_9MgCl (1)	C_2H_5	Cl	26	1	**12a**	91
36	**10a**	$LiAl[N(CH_3)_2]_4$[m] (1)	C_2H_5	Cl	−20 to 0	3	**12a**	97[c]
37	**10b**·HCl	*t*-C_4H_9MgCl (2)	C_2H_5	Cl	26	2	**12b**	76[c]
38	**10b**·HCl	$LiAl[N(CH_3)_2]_4$[g] (1)	C_2H_5	Cl	−40 to 0	20	**12b**	74[c]
39	**10e**	*t*-C_4H_9MgCl (2)	C_2H_5	Cl[l]	26	1	**12e**	91[c]
40	**10e**	$LiAl[N(CH_3)_2]_4$[m] (1)	C_2H_5	Cl	−20	6	**12e**	87[c]

[a] Unless otherwise noted, the reaction was carried out in THF by using 1 equiv of the metalating agent and 1.1–1.2 equiv of the phosphorylating agent. [b] Determined by ^1H NMR analysis, unless otherwise stated. [c] Isolated yield. [d] Mesityllithium. [e] (2,6-Dimethoxyphenyl)lithium. [f] Three equivalents of *p*-nitrophenol was added. [g] Two equivalents of *o*-chlorophenol was added. [h] Diisobutylaluminum hydride. [i] N^4,O-Diphosphorylated nucleoside (17%) was obtained as byproduct. [j] The major products were N^4-phosphorylated cytidine (30%) and N^4,O-diphosphorylated derivative (19%). [k] The reaction was achieved in DMF. [l] The reaction was performed by use of 1.4 equiv of the phosphorylating agent. [m] Three equivalents of *o*-chlorophenol was added.

Scheme II

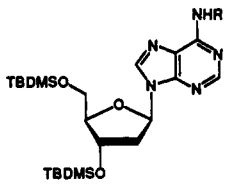


nucleoside anions as magnesium salts. The ratio of the magnesium amide (or iminoxide) **14** to the magnesium alkoxide **13** in the equilibrium mixture was determined by colorimetric analysis and can be calculated by the equation of $(\epsilon_{HB} - \epsilon_{obs})/(\epsilon_{obs} - \epsilon_{B^-})$, where ϵ_{HB}, ϵ_{B^-}, and ϵ_{obs} are molar extinction coefficient of the nucleoside-base chromophore of the original nucleoside, the derivative with the completely deprotonated base moiety, and the equilibrium mixture, respectively. The 3′-free nucleosides **5a–d** were employed for the determination of the ϵ_{HB}. The ϵ_{B^-} value was obtained by the UV spectrum of a mixture of 1 equiv of *tert*-butylmagnesium chloride and **17**, **23**, 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine, or -2′-deoxyguanosine, whereas ϵ_{obs} was measured using a mixture of equimolar amounts of **5a–d** and the Grignard reagent. The equilibrium ratios of the magnesium amide (or iminoxide)/the magnesium alkoxide are 2.0/98.0 in adenosine **5a** and 6.3/93.7 in cytidine **5b**, indicating the predominant alkoxide formation in adenosine and cytidine. On the other hand, preferential formation of the magnesium iminoxide was observed with guanosine (98.7/1.3 in **5c**) and thymidine (98.0/2.0 in **5d**). The selectivity in the formation of oxy or nitrogen anions is controlled by the acidity of the functional groups, i.e., guaninyl 1-H and thyminyl or uracil 3-H (pK_a ca. 9) > OH of sugar (17–18) > adeninyl 6-NH_2 and cytosinyl 4-NH_2 (ca. 20[13]). Thus the nucleosides are divided into two groups: group I includes adeninyl and cytosinyl nucleosides having no protons more acidic than hydroxyl protons and group II

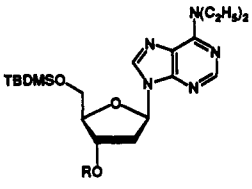
(13) Dolman, D.; Stewart, R. *Can. J. Chem.* **1967**, *45*, 911.

consists of guaninyl, thyminyl, and uracil nucleosides having highly acidic protons in the nucleobase moieties.

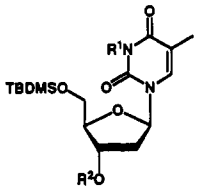
To compare their chemical behavior we chose protected adenosine derivatives, **17** and **20**, and protected thymidines, **23** and **24**, as *monofunctional* group I and group II nucleosides, respectively. The relative reactivities of the magnesium alkoxide (k_o) to the amide or iminoxide (k_n) were determined by the product distribution in competitive phosphorylation of the *O*-Mg species, **21** and **25**, vs *N*-Mg species, **18** and **27**, toward $(C_2H_5O)_2POCl$. Phosphorylation of a mixture of equimolar amounts of the Mg amide and alkoxide, **18** and **21**, with 0.1, 0.2, and 0.4 equiv of $(C_2H_5O)_2POCl$ gave a 1:24–1:29 mixture of the phosphoramidate **19** and the phosphate **22**, indicating that the magnesium alkoxides are more reactive than the magnesium amides. Therefore phosphorylation of group I nucleosides (deoxyadenosine and deoxycytidine), activated mainly as magnesium alkoxides, selectively gives the corresponding phosphate. The minor and less reactive Mg amides are equilibrated to form the more reactive alkoxides during the reaction. Overall, as illustrated in Scheme II, exclusive O-phosphorylation is achieved via path a. This is consistent with the calculated O-selectivity, $K(k_o/k_n) = (98/2)(27/1) = 1200$.



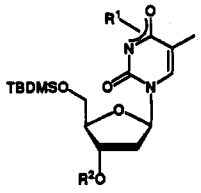
17, R = H
18, R = MgCl
19, R = $(C_2H_5O)_2PO$



20, R = H
21, R = MgCl
22, R = $(C_2H_5O)_2PO$



23, R^1 = H; R^2 = TBDMS
24, R^1 = CH_3; R^2 = H
25, R^1 = CH_3; R^2 = MgCl
26, R^1 = CH_3; R^2 = $(C_2H_5O)_2PO$



27, R^1 = MgCl; R^2 = TBDMS
28, R^1 = $(C_2H_5O)_2PO$; R^2 = H
29, R^1 = $(C_2H_5O)_2PO$; R^2 = TBDMS

Chemoselectivity in phosphorylation of group II nucleosides is not straightforward. Competitive reaction of a 1:50 mixture of the Mg salts **25** and **27** with 0.1 equiv of $(C_2H_5O)_2POCl$ led to formation of the phosphate **26** in ≥75% yield within 0.5 min. As is observed with group I nucleoside, the magnesium alkoxide reacts much faster than the magnesium iminoxide ($k_o/k_n \geq 150$). However, the Mg iminoxide is thermodynamically more stable than the alkoxide. The calculated O-selectivity is estimated to be $K(k_o/k_n) = (2/98)(\geq 150/1) \geq 3.1$. In addition, the phosphoryl iminoxide (or phosphorimidate) is not thermodynamically stable, undergoing isomerization to the phosphate. When 1 equiv of the Grignard base was employed as the activator of nucleoside **5d**, the reactions via path a and path b occurred competitively; the major, less reactive magnesium amide and the minor, more reactive magnesium alkoxide were simultaneously phosphorylated under the reaction conditions to give a mixture of **7d** (^{31}P NMR, δ −5.9 ppm) and **28** (a regioisomeric mixture, δ −8.3 and −9.3 ppm) as the initial product (see Figure 1). Conversion of **28** to **7d** via path c then took place slowly by some intermolecular process giving **7d** as a single phosphorylated product after 96 h. Thus, the desired O-phosphorylation was eventually accomplished even with 1 equiv of *tert*-butylmagnesium chloride. In the reaction of **5d**, the phosphate **6d** or **7d** was obtained rapidly with 2 equiv of the Grignard reagent. Activation of both hydroxyl and imide as the Mg salts, coupled with the higher reactivity of the magnesium alkoxide, provides a simple solution to the selectivity problem.

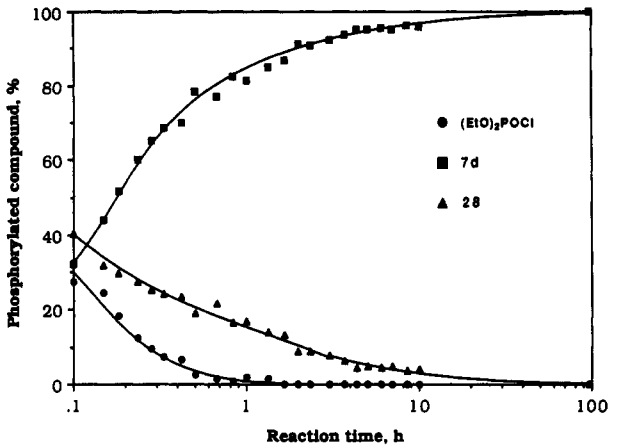


Figure 1. Profile of the Grignard reagent (1 equiv)-assisted phosphorylation of **5d** (1 equiv) using diethyl phosphorochloridate (1 equiv) monitored by ^{31}P NMR at 25 °C in THF (concentration = 0.06 M).

Conclusions

A facile O-selective phosphorylation of nucleosides without any N-protection has been achieved on the basis of hydroxyl activation. Use of an organomagnesium reagent as the activator is most effective. This reaction is highly appropriate for large-scale synthesis of nucleoside phosphates because of its high chemoselectivity, operational simplicity, and the low price of the reagents. The mechanism leading to the O-phosphorylation depends on the nucleoside structure and the amount of the activator employed. Utility of this method has been demonstrated by the synthesis of 2′-5′-linked oligoadenylates (2-5As) and their analogues.[14]

Experimental Section

General. Spectra measurement was taken with instruments described in the literature.[14] Fast atom bombardment mass spectra (FABMS) were measured at the Faculty of Agriculture, Nagoya University. Solvents employed for reactions and chromatography were purified by the standard methods.[14] Reactions requiring anhydrous conditions were conducted in a well-baked vessel under an argon atmosphere. The ^1H NMR analysis of yields of products was performed by comparing the relative intensity of the signal of 1,1,2,2-tetrachloroethane as an internal standard with that of the signal due to the phosphorylated product: H-2 for adenine nucleotides and H-3′ for other 3′-nucleotides, respectively. For column chromatography, E.

(14) Noyori, R.; Uchiyama, M.; Nobori, T.; Hirose, M.; Hayakawa, Y. *Aust. J. Chem.* **1992**, *45*, 205.

Merck Kieselgel-60 (70–230 mesh), deactivated by adding 6% of water, was used.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxyadenosine (**5a**), 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**5b**), 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine (**5c**), 5′-*O*-(*tert*-butyldimethylsilyl)thymidine (**5d**), 3′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**8a**), 3′-*O*-(*tert*-butyldimethylsilyl)thymidine (**8d**), 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine (**17**), 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)thymidine (**23**), and 5′-*O*-(*tert*-butyldimethylsilyl)-*N*(3)-methylthymidine (**24**) were prepared by known procedures.[15] *N*(3)-Methylthymidine was prepared by the reported method.[16] Commercially available 2′,3′-*O*-isopropylideneadenosine (**10a**), 2′,3′-*O*-isopropylidenecytidine (**10b**) hydrochloride, and 2′,3′-*O*-isopropylideneuridine (**10c**) were used after drying at 50–60 °C over P_2O_5 under reduced pressure or by azeotropic removal of water with pyridine. An ether solution of methyllithium,[17] a solution of mesityllithium in a THF–ether–pentane mixture,[18] a THF solution of (2,6-dimethoxyphenyl)-lithium,[19] and a solution of *tert*-butylmagnesium chloride in THF[20] were prepared according to the literature methods. A THF solution of $LiAl[N(CH_3)_2]_4$ and $Al[N(CH_3)_2]_3$ were prepared by the reported procedure.[21] A commercially supplied hexane solution of *n*-butyllithium, a pentane solution of *tert*-butyllithium, and a hexane solution of diisobutylaluminum hydride were used after dilution with dry hexane. The active organolithiums in the stock solutions were titrated by known methods.[22] Total bases of the solutions were determined by acid–base titration after hydrolysis. Concentration of the Grignard reagent was determined by the Gilman method.[23] Concentrations of these aluminum compounds were determined by a combination of titration of total base after hydrolysis and titration of dimethylamine after methanolysis. Diethyl *p*-nitrophenyl phosphate and bis(*o*-chlorophenyl) *p*-nitrophenyl phosphate were obtained by the known procedure.[24] The pK_a values of 17 (adeninyl 6-NH_2) and 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)cytidine (cytosinyl 4-NH_2) were determined by the method of Dolman and Stewart.[13]

Reaction of Bis(*o*-chlorophenyl) Phosphorochloridate with Nucleoside 5a in Pyridine. Bis(*o*-chlorophenyl) phosphorochloridate (74.3 mg, 0.22 mmol) was added to a solution of **5a** (73.0 mg, 0.20 mmol) in pyridine (2 mL) at 0 °C. The mixture was stirred at 0 °C for 11 h and quenched by addition of water (0.2 mL). The mixture was evaporated to give a liquid, which was then coevaporated with benzene (1 mL × 3). The oily material was dissolved in a mixture of dichloromethane (20 mL) and saturated $NaHCO_3$ solution (20 mL). The mixture was extracted with dichloromethane (20 mL, 5 mL × 2), dried, and concentrated to give a gum (0.14 g). The crude product was subjected to silica gel column chromatography. Elution with a 1:1:50 to 1:1:30 mixture of methanol, ethyl acetate, and chloroform afforded bis(*o*-chlorophenyl) 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine 3′-phosphate (**6a**) (18.2 mg, 14%), mp 114–115 °C, and 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine bis(o-chlorophenyl) N^6-phosphoramidate (34.7 mg, 26%). **6a**: IR 3490, 3400, 1640, 1630, 1590, 1290 cm^{-1}; UV λ_{max} 260 nm (ϵ 16 700); ^{1}H NMR 0.08 (s, 6 H, $(CH_3)_2Si$), 0.89 (s, 9 H, *t*-C_4H_9Si), 2.87 (m, 2 H, 2 H-2′), 3.90 (d, 2 H, J = 3.1 Hz, 2 H-5′), 4.43 (m, 1 H, H-4′), 5.55 (m, 1 H, H-3′), 5.87 (br s, 2 H, NH_2), 6.52 (t, 1 H, J = 7.0 Hz, H-1′), 7.0–7.6 (m, 8 H, 2 ClC_6H_4), 8.11 (s, 1 H, H-2), 8.34 (s, 1 H, H-8); ^{13}C NMR −6.0, −5.9, 17.8, 25.4, 39.2 (d, J = 4 Hz), 62.7, 80.6 (d, J = 4 Hz), 83.8, 85.5 (d, J = 7 Hz), 119.4, 121.9 (d, J = 2 Hz), 125.0 (d, J = 7 Hz), 126.2, 127.6, 130.3, 138.0, 145.8 (d, J = 7 Hz), 149.1, 152.6, 155.6. Anal. Calcd for $C_{28}H_{34}Cl_2N_5O_6PSi$: C, 50.45; H, 5.14; N, 10.51. Found: C, 50.22; H, 5.15; N, 10.54. The deoxyadenosine N^6-phosphoramidate: IR 3380, 1650, 1640, 1280 cm^{-1}; UV λ_{max} 263 nm; ^{1}H NMR 0.05 (s, 6 H, $(CH_3)_2Si$), 0.83 (s, 9 H, *t*-C_4H_9Si), 2.5–2.7 (m, 2 H, 2 H-2′), 3.83 (m, 2 H, 2 H-5′), 4.0–4.2 (m, 1 H, H-4′), 4.5–4.7 (m, 1 H, H-3′), 6.46 (t, 1 H, J = 7.0 Hz, H-1′), 7.0–7.6 (m, 9 H, 2 ClC_6H_4, NHPO), 8.30 (s, 1 H, H-2), 8.43 (s, 1 H, H-8).

Phosphorylation Promoted by *tert*-Butylmagnesium Chloride. Diethyl 5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxyadenosine 3′-Phosphate (7a). To a solution of the nucleoside **5a** (1.46 g, 4.00 mmol) in THF (40 mL) was added dropwise at 25 °C a 0.29 M solution of *tert*-butylmagnesium chloride (13.9 mL, 4.10 mmol) in THF. After stirring for 5 min, diethyl phosphorochloridate (8.28 mg, 4.80 mmol) was added, and stirring was continued for 1 h. The mixture was diluted with dichloromethane (200 mL), quenched with saturated NH_4Cl solution (250 mL), and then extracted with dichloromethane (50 mL × 2). The combined organic layers were washed with brine (50 mL), dried, and evaporated to give a gum. The ^{1}H NMR spectrum of the crude product showed that **7a** was produced in 94% yield. Column chromatography on silica gel eluted with a 1:40 to 1:30 mixture of methanol and chloroform gave **7a** (1.81 g, 91%): IR 3540, 3405, 1640, 1625, 1580, 1290 cm^{-1}; UV λ_{max} 260 nm (ϵ 14 500); ^{1}H NMR 0.04 (s, 6 H, $(CH_3)_2Si$), 0.85 (s, 9 H, *t*-C_4H_9Si), 1.31 (dt, 6 H, J = 0.9, 7.2 Hz, $(CH_3CH_2O)_2PO$), 2.6–2.8 (m, 2 H, 2 H-2′), 3.90 (d, 2 H, J = 9 Hz, 2 H-5′), 3.9–4.3 (m, 5 H, H-4′, $(CH_3CH_2O)_2$-PO), 5.0–5.2 (m, 1 H, H-3′), 6.21 (br s, 2 H, NH_2), 6.45 (t, 1 H, J = 6.8 Hz, H-1′), 8.07 (s, 1 H, H-2), 8.29 (s, 1 H, H-8); ^{13}C NMR −6.0, −5.9, 15.7 (d, J = 7 Hz), 17.8, 25.4, 39.0 (d, J = 4 Hz), 62.8, 63.6 (d, J = 7 Hz), 77.6 (d, J = 6 Hz), 83.8, 85.6 (d, J = 6 Hz), 119.3, 138.0, 149.1, 152.5, 155.5; FABMS *m/z* 502 [(MH)$^+$]. Anal. Calcd for $C_{20}H_{36}N_5O_6PSi$: C, 47.89; H, 7.23; N, 13.96. Found: C, 47.86; H, 7.25; N, 13.63.

Diethyl 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine 3′-phosphate (7b): 95% (^{1}H NMR assay); IR 3540, 3400, 1660, 1640, 1600, 1260 cm^{-1}; UV λ_{max} 272 nm (ϵ 9900); ^{1}H NMR 0.10 (s, 6 H, $(CH_3)_2Si$), 0.90 (s, 9 H, *t*-C_4H_9Si), 1.34 (dt, 6 H, J = 1.1, 7.1 Hz, $(CH_3CH_2O)_2PO$), 1.9–2.3, 2.6–2.8 (2 m's, 2 H, 2 H-2′), 3.89 (m, 2 H, 2 H-5′), 4.11 (dq, 4 H, J = 7.3, 7.1 Hz, $(CH_3CH_2O)_2PO$), 4.27 (m, 1 H, H-4′), 4.92 (m, 1 H, H-3′), 5.67 (d, 1 H, J = 7.4 Hz, H-5), 6.00 (br s, 2 H, NH_2), 6.38 (dd, 1 H, J = 1.8, 11.7 Hz, H-1′), 7.90 (d, 1 H, J = 7.4 Hz, H-6); ^{13}C NMR −6.2, −6.1, 15.4 (d, J = 7 Hz), 17.6, 25.5, 39.4 (d, J = 4 Hz), 62.5, 63.4 (d, J = 8 Hz), 77.3 (d, J = 6 Hz), 85.0 (d, J = 8 Hz), 85.2, 94.7, 139.4, 155.3, 165.5; FABMS *m/z* 478 [(MH)$^+$]. Anal. Calcd for $C_{19}H_{36}N_3O_7PSi$: C, 47.79; H, 7.60; N, 8.80. Found: C, 47.66; H, 7.63; N, 8.76.

Diethyl 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyguanosine 3′-phosphate (7c): 89%; IR 3500, 3400, 1690, 1630, 1610, 1255 cm^{-1}; UV λ_{max} 255 nm (ϵ 13 400); ^{1}H NMR 0.06 (s, 6 H, $(CH_3)_2Si$), 0.90 (s, 9 H, *t*-C_4H_9Si), 1.37 (t, 6 H, J = 7.0 Hz, $(CH_3CH_2O)_2PO$), 2.5–2.8 (m, 2 H, 2 H-2′), 3.7–3.9 (m, 2 H, 2 H-5′), 4.0–4.4 (m, 5 H, $(CH_3CH_2O)_2PO$, H-4′), 5.10 (m, 1 H, H-3′), 6.25 (t, 1 H, J = 7.0 Hz, H-1′), 6.47 (br s, 2 H, NH_2), 7.88 (s, 1 H, H-8), 11.9 (br s, 1 H, NH); ^{13}C NMR −7.2, −7.1, 14.6 (d, J = 7 Hz), 17.2, 24.5, 37.7 (d, J = 5 Hz), 62.2, 63.7 (d, J = 6 Hz), 77.4 (d, J = 6 Hz), 83.0 (d, J = 6 Hz), 85.1 (d, J = 6 Hz), 115.9, 135.2, 150.5, 153.2, 157.4; FABMS *m/z* 518 [(MH)$^+$]. Anal. Calcd for $C_{20}H_{36}N_5O_7$-PSi: C, 46.41; H, 7.01; N, 13.53. Found: C, 45.49; H, 6.79; N, 13.27.

Diethyl 5′-*O*-(*tert*-butyldimethylsilyl)thymidine 3′-phosphate (7d): 91%; mp 70–74 °C; IR 3380, 1710, 1695, 1260 cm^{-1}; UV λ_{max} 266 nm (ϵ 11 200); ^{1}H NMR 0.03 (s, 6 H, $(CH_3)_2Si$), 0.77 (s, 9 H, *t*-C_4H_9Si), 1.20 (t, 6 H, J = 7.0 Hz, $(CH_3CH_2O)_2PO$), 1.76 (s, 3 H, C(5)CH_3), 2.1–2.7 (m, 2 H, 2 H-2′), 3.47 (br s, 2 H, 2 H-5′), 3.8–4.2 (m, 5 H, H-4′, $(CH_3CH_2O)_2PO$), 4.84 (m, 1 H, H-3′), 6.25 (dd, 1 H, J = 6.0, 9.0 Hz, H-1′), 7.34 (s, 1 H, H-6), 10.15 (br s, 1 H, NH); ^{13}C NMR −6.0, −5.9, 12.0, 15.7, 17.9, 25.5, 38.9 (d, J = 6 Hz), 62.8, 63.7 (d, J = 7 Hz), 77.7 (d, J = 6 Hz), 84.1, 85.3 (d, J = 4 Hz), 110.6, 134.4, 150.2, 163.7; FABMS *m/z* 493 [(MH)$^+$]. Anal. Calcd for $C_{20}H_{37}N_2O_8PSi$: C, 48.76; H, 7.59; N, 5.69. Found: C, 48.78; H, 7.45; N, 5.64.

Diethyl 2′,3′-*O*-isopropylideneadenosine 5′-phosphate (12a): 91%; mp 139–141 °C; IR 3490, 3400, 1640, 1630, 1590, 1290 cm^{-1}; UV λ_{max} 259 nm (ϵ 17 300); ^{1}H NMR 1.28 (m, 6 H, $(CH_3CH_2O)_2PO$), 1.40, 1.63 (2 s's, 6 H, $C(CH_3)_2$), 3.7–4.6 (m, 7 H, $(CH_3CH_2O)_2PO$, H-2′, H-3′, H-4′), 5.10 (dd, 1 H, J = 3.6, 7.5 Hz, H-5′), 5.40 (dd, 1 H, J = 3.0, 7.5 Hz, H-5′), 5.68 (br s, 2 H, NH_2), 6.14 (d, 1 H, J = 2.5 Hz, H-1′), 7.94 (s, 1 H, H-2), 8.34 (s, 1 H,

(15) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799.
(16) Zemlicka, J. *Collect. Czech. Chem. Commun.* **1970**, *35*, 3572.
(17) Wittig, G.; Hesse, A. *Org. Synth.* **1970**, *50*, 67.
(18) Neumann, H.; Seebach, D. *Tetrahedron Lett.* **1976**, 4839.
(19) Lambert, G. J.; Duffley, R. P.; Dalzell, H. C.; Razdan, R. K. *J. Org. Chem.* **1982**, *47*, 3350.
(20) Fild, M.; Stelzer, O.; Schmutzler, R. *Inorg. Synth.* **1973**, *14*, 5.
(21) Ruff, J. K. *J. Am. Chem. Soc.* **1961**, *83*, 2835.
(22) Korfron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, *41*, 1879.
(23) Crompton, T. R. *Chemical Analysis of Organometallic Compounds*; Academic: New York, 1973; Vol. 1, pp 67–88.
(24) Cherbuliez, E. In *Organic Phosphorus Compounds*; Kosolapoff, G. M., Maier, L., Eds.; Wiley: New York, 1973; Vol. 6, Chapter 15.

H-8); ^{13}C NMR 15.6 (d, J = 7 Hz), 25.0, 26.7, 63.7 (d, J = 6 Hz), 66.4 (d, J = 5 Hz), 81.1, 83.8, 85.0 (d, J = 8 Hz), 90.5, 114.1, 119.6, 139.1, 148.8, 152.8, 155.7. Anal. Calcd for $C_{17}H_{26}N_5O_7P$: C, 46.05; H, 5.91; N, 15.80. Found: C, 45.90; H, 5.82; N, 15.76.

Diethyl 2′,3′-*O*-isopropylidenecytidine 5′-phosphate (12b): 76%; IR 3500, 3400, 1670, 1650, 1600, 1260 cm^{-1}; UV λ_{max} 269 (ϵ 7200), 240 nm (ϵ 7700); ^{1}H NMR 1.12 (m, 9 H, CH_3C, $(CH_3CH_2O)_2PO$), 1.52 (s, 3 H, CH_3C), 3.9–4.4 (m, 7 H, $(CH_3CH_2O)_2PO$, H-2′, H-3′, H-4′), 5.02 (dd, 2 H, J = 7.2, 19.2 Hz, 2 H-5′), 5.46 (br s, 1 H, H-1′), 6.00 (d, 1 H, J = 7.2 Hz, H-5), 7.29 (d, 1 H, J = 7.2 Hz, H-6), 8.30 (br s, 2 H, NH_2); ^{13}C NMR 16.1 (d, J = 7 Hz), 25.3, 27.1, 64.1 (d, J = 7 Hz), 67.3 (d, J = 6 Hz), 82.2, 84.9, 86.7 (d, J = 8 Hz), 95.7, 97.6, 113.7, 143.9, 155.6, 166.7. Anal. Calcd for $C_{16}H_{26}N_3O_8P$: C, 45.82; H, 6.25; N, 10.02. Found: C, 45.88; H, 6.17; N, 9.87. Anal. Calcd for $C_{16}H_{26}N_3O_8P$: C, 45.82; H, 6.25; N, 10.02. Found: C, 45.88; H, 6.17; N, 9.87.

Diethyl 2′,3′-*O*-isopropylideneuridine 5′-phosphate (12e): 91%; IR 3390, 1730, 1695, 1270 cm^{-1}; UV λ_{max} 259 nm (ϵ 9200); ^{1}H NMR 1.35 (m, 9 H, CH_3C, $(CH_3CH_2O)_2PO$), 1.58 (s, 3 H, CH_3C), 4.0–4.2 (m, 7 H, $(CH_3CH_2O)_2PO$, H-2′, H-3′, H-4′), 4.88 (m, 2 H, 2 H-5′), 5.70 (d, 1 H, J = 7.5 Hz, H-5), 5.78 (d, 1 H, J = 1.2 Hz, H-1′), 7.38 (d, 1 H, J = 7.5 Hz, H-6), 9.52 (br s, 1 H, NH); ^{13}C NMR 15.8 (d, J = 7 Hz), 25.0, 26.8, 63.8 (d, J = 6 Hz), 66.5 (d, J = 6 Hz), 80.4, 84.1, 84.6 (d, J = 8 Hz), 93.4, 102.3, 114.3, 141.5, 149.9, 163.3. Anal. Calcd for $C_{16}H_{25}N_2O_9P$: C, 45.72; H, 5.99; N, 6.66. Found: C, 45.81; H, 6.20; N, 6.28.

Diethyl 5′-*O*-(*tert*-butyldimethylsilyl)-N^6,N^6-diethyl-2′-deoxyadenosine 3′-phosphate (22): 91%; HPLC (λ = 260 nm, 40 °C, a 1:10 mixture of water and methanol) t_R = 7.9 min; IR 3650, 3414, 1592, 1563, 1261 cm^{-1}; UV λ_{max} 277 nm (ϵ 18 900); ^{1}H NMR 0.09 (s, 6 H, $(CH_3)_2Si$), 0.90 (s, 9 H, t-C_4H_9Si), 1.28 (t, 6 H, J = 7.0 Hz, $(CH_3CH_2)_2N$), 1.36 (dt, 6 H, J = 0.9, 7.2 Hz, $(CH_3CH_2O)_2PO$), 2.74 (m, 2 H, 2 H-2′), 3.87 (m, 6 H, $(CH_3CH_2)_2N$, 2 H-5′), 3.9–4.2 (quintet, 4 H, J = 7.2 Hz, $(CH_3CH_2O)_2PO$), 4.32 (m, 1 H, H-4′), 5.13 (m, 1 H, H-3′), 6.49 (t, 1 H, J = 6.2 Hz, H-1′), 7.97 (s, 1 H, H-2), 8.31 (s, 1 H, H-8). Anal. Calcd for $C_{24}H_{44}N_5O_6PSi$: C, 51.69; H, 7.95; N, 12.56. Found: C, 51.75; H, 8.13; N, 12.48.

Diethyl 5′-*O*-(*tert*-butyldimethylsilyl)-*N*(3)-methylthymidine 3′-phosphate (26): 92%; HPLC (λ = 260 nm, 40 °C, a 1:5 mixture of water and methanol) t_R = 8.7 min; IR 3672, 3444, 1701, 1670, 1641, 1473, 1285 cm^{-1}. UV λ_{max} 266 nm (ϵ 10 500). ^{1}H NMR 0.11, 0.12 (2 s's, 6 H, $(CH_3)_2Si$), 0.91 (s, 9 H, t-C_4H_9Si), 1.35 (dt, 6 H, J = 1.2, 7.2 Hz, $(CH_3CH_2O)_2PO$), 1.93 (d, 3 H, J = 1.2 Hz, C(5)CH_3), 2.1–2.3, 2.5–2.6 (m, 2 H, 2 H-2′), 3.34 (s, 3 H, CH_3N), 3.85 (dd, 1 H, J = 2.1, 11.7 Hz, H-5′), 3.91 (dd, 1 H, J = 2.4, 11.7 Hz, H-5′), 4.13 (dq, 4 H, J = 7.4, 7.2 Hz, $(CH_3CH_2O)_2PO$), 4.27 (m, 1 H, H-4′), 4.96 (m, 1 H, H-3′), 6.39 (dd, 1 H, J = 5.3, 8.7 Hz, H-1′), 7.46 (d, 1 H, J = 1.2 Hz, 6-H); ^{31}P NMR (C_6D_6–THF, 1:3) -1.7, (C_6D_6–THF, 1:3, with 1 equiv of $MgCl_2$) -5.4. Anal. Calcd for $C_{20}H_{39}N_2O_8PSi$: C, 48.57; H, 7.95; N, 5.66. Found: C, 48.77; H, 7.68; N, 5.66.

3′,5′-Bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine diethyl N^6-Phosphoramidate (19): 93%; HPLC (λ = 260 nm, 40 °C, a 1:10 mixture of water and methanol) t_R = 14.1 min; IR 3686, 3384, 1609, 1257 cm^{-1}; UV λ_{max} 260 nm (ϵ 16 000); ^{1}H NMR 0.08, 0.09 (2 s's, 12 H, 2 $(CH_3)_2Si$), 0.91, 0.92 (2 s's, 18 H, 2 t-C_4H_9Si), 1.36 (t, 6 H, J = 7.0 Hz, $(CH_3CH_2O)_2PO$), 2.45, 2.70 (2 m's, 2 H, 2 H-2′), 3.77 (dd, 1 H, J = 3.1, 10.9 Hz, H-5′), 3.87 (dd, 1 H, J = 4.4, 11.3 Hz, H-5′), 4.03 (m, 1 H, H-4′), 4.2–4.4 (m, 4 H, $(CH_3CH_2)_2PO$), 4.63 (m, 1 H, H-3′), 6.46 (t, 1 H, J = 6.4 Hz, H-1′), 7.49 (br s, 1 H, NHPO), 8.31 (s, 1 H, H-2), 8.60 (s, 1 H, H-8). Anal. Calcd for $C_{28}H_{50}N_5O_6PSi_2$: C, 50.71; H, 8.34; N, 11.33. Found: C, 50.59; H, 8.34; N, 11.33.

Lithium Alkoxide-Mediated Phosphorylation. Bis(*o*-chlorophenyl) 5′-*O*-(*tert*-Butyldimethylsilyl)-2′-deoxyadenosine 3′-Phosphate (6a). The phosphorylation using *tert*-butyllithium as a strong base is representatively described. To a THF (30 mL) solution of the nucleoside **5a** (1.07 g, 2.94 mmol) was added at −78 °C a 0.588 M solution of *tert*-butyllithium in a 1:1 mixture of pentane and hexane (5.0 mL, 2.94 mmol) over 30 min. After being stirred for 1.5 h, to the resulting suspension was added bis(*o*-chlorophenyl) phosphorochloridate (1.03 g, 3.06 mmol) in THF (8.7 mL) over 15 min, and the mixture was stirred at the same temperature for additional 1.25 h. The reaction mixture was quenched with brine (20 mL) and extracted with dichloromethane (100 mL, 50 mL, 30 mL × 2). The combined organic extracts were washed with brine (10 mL) and dried. Evaporation gave a colorless gum (2.11 g), whose ^{1}H NMR analysis indicated that **6a** was formed in 95% yield. The crude material was subjected to silica gel column chromatography and eluted with a 1:10:40 mixture of methanol, acetone, and dichloromethane. Concentration of the eluent gave **6a** (1.79 g, 91%) as crystals.

Organoaluminum-Assisted Phosphorylation. Phosphorylation of Lithium Aluminate-Type Alkoxide of Nucleoside [LiAl(OAr)$_3$(ONu)]. The procedure for the reaction of 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine (**5b**) using *p*-nitrophenol as ArOH is representative. To a 0.28 M THF solution of lithium tetrakis(dimethylamino)aluminate[21] (12.5 mL, 3.47 mmol) in an argon-filled vessel was added at 0 °C dropwise through a stainless cannula a THF (18 mL) solution of the nucleoside **5b** (1.18 g, 3.47 mmol) in a separated, argon-filled flask. After the mixture was stirred at the same temperature for 10 min, to the mixture was added *p*-nitrophenol (1.45 g, 10.4 mmol) in THF (10 mL) at 0 °C. The resulting solution was stirred at 20 °C for 1 h and evaporated with a vacuum pump to give a foam, which was dissolved in THF (16 mL). To the resulting mixture was added diethyl phosphorochloridate (670 mg, 3.88 mmol) in THF (4 mL) at 0 °C. The mixture was stirred at the same temperature for 6 h and poured into a 0.5 M NaOH solution (80 mL) and extracted with dichloromethane (80 mL × 2, 40 mL × 2). The combined organic layers were washed with brine (30 mL), dried, and concentrated to give a gum. The ^{1}H NMR spectrum of the crude product showed that diethyl 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine 3′-phosphate (**7b**) was formed in 95% yield. Silica gel (50 g) column chromatography (a 1:30 to 1:20 mixture of methanol and chloroform) afforded the analytical sample (1.42 g, 86%).

UV Spectra Measurement for Determination of the Ratios of Magnesium Alkoxide and Amide. The nucleoside **5a–d**, **17**, **23**, 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-2′-deoxycytidine, or the corresponding 2′-deoxyguanosine analogue (0.100 mmol) and THF (35 mL) were charged in a 50-mL measuring flask, which was well dried and filled with argon. To this solution was added a 0.190 M solution of *tert*-butylmagnesium chloride in THF (0.530 mL, 0.100 mmol) at 25 °C. After shaking, THF was added to bring it up to 50 mL, and the mixture was sonicated until determined to be homogeneous. A 0.50-mL portion of this solution was transferred into a 10-mL measuring flask to adjust the volume to 10 mL by addition of dioxane. This mixed solvent was necessary to obtain the spectra; THF alone as the solvent interfered by its end absorption. These sample were transferred by a stainless tube and measured in a quartz cell which was well dried, filled with argon, and equipped with a rubber septum. The spectra of original nucleosides **5a–d** was measured in the same mixed solvent, omitting addition of the Grignard reagent.

3′-*O*-Benzoyl-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyadenosine. To a THF (50 mL) solution of benzoyl chloride (7.33 g, 52.1 mmol) was added a mixture of 1*H*-tetrazole (4.02 g, 57.4 mmol) and triethylamine (7.92 g, 78.3 mmol) in THF (100 mL) at 0 °C. After 2 h of stirring, the resulting precipitates were removed through a short column packed with Celite 545. Evaporation excluding moisture afforded an oil, to which was added a mixture of the nucleoside **5a** (9.53 g, 26.1 mmol) and triethylamine (5.27 g, 52.1 mmol) in THF (100 mL) at 25 °C. The mixture was stirred for 1 h and poured into a 1:1 mixture of ethyl acetate and brine (500 mL). The aqueous layer was extracted with ethyl acetate (100 mL × 3). The combined organic layers were washed with brine and dried. Concentration gave a foam, which was recrystallized from a mixture of ethyl acetate and hexane to produced the title compound (9.56 g, 78%): IR (KBr) 3590, 3320, 3164, 1718, 1676, 1603 cm^{-1}; UV λ_{max} 260 (ϵ 14 500), 232 nm (ϵ 14 500); ^{1}H NMR 0.15 (s, 6 H, $(CH_3)_2Si$), 0.94 (s, 9 H, t-C_4H_9Si), 2.79 (dd, 2 H, J = 3.9, 7.2 Hz, 2 H-2′), 3.90 (dd, 2 H, J = 3.0, 10.2 Hz, 2 H-5′), 4.38 (m, 1 H, H-4′), 5.67 (m, 1 H, H-3′), 5.77 (br s, 2 H, NH_2), 6.45 (t, 1 H, J = 7.2 Hz, H-1′), 7.48 (m, 2 H, benzoyl), 7.60 (m, 1 H, benzoyl), 8.08 (m, 2 H, benzoyl), 8.24 (s, 1 H, H-2), 8.36 (s, 1 H, H-8). Anal. Calcd for $C_{23}H_{31}N_5O_4Si$: C, 58.83; H, 6.65; N, 14.91. Found: C, 58.61; H, 6.78; N, 14.86.

5′-*O*-(*tert*-Butyldimethylsilyl)-N^6,N^6-diethyl-2′-deoxyadenosine (20). According to the reported procedure,[25] chlorination at the 6-position of adenine was performed using the above obtained nucleoside (7.55 g, 16.1 mmol) to give the desired intermediate (3.91 g, 50%) as a gum: IR 1719, 1591, 1562 cm^{-1}; UV λ_{max} 265 (ϵ 10 500), 231 nm (ϵ 16 500); ^1H NMR 0.13 (s, 6 H, $(CH_3)_2Si$), 0.91 (s, 9 H, t-C_4H_9Si), 2.7–2.9 (m, 2 H, 2 H-2′), 3.90 (m, 2 H, 2 H-5′), 4.42 (m, 1 H, H-4′), 5.67 (m, 1 H, H-3′), 6.45 (dd, 1 H, J = 6.0, 6.4 Hz, H-1′), 7.45 (m, 2 H, benzoyl), 7.60 (m, 1 H, benzoyl), 8.07 (m, 2 H, benzoyl), 8.59 (s, 1 H, H-2), 8.74 (s, 1 H, H-8). Diethylamine (20 mL) was added to the aforementioned product and stirred at 25 °C for 3 h and evaporated to dryness. The gummy residue (4.85 g) was dissolved in methanol (80 mL). To this solution was added a 0.5 M solution of sodium hydroxide (40 mL) at 0 °C. The mixture was stirred at the same temperature for 1.5 h and poured into a 1:1 mixture of dichloromethane and saturated ammonium chloride solution (400 mL). The aqueous layer was extracted with dichloromethane (100 mL × 3). The combined organic layers were washed with brine (50 mL) and dried. The residue (4.50 g) after evaporation was subjected to silica gel (160 g) column chromatography. Elution with a 1:30 to 1:20 mixture of methanol and chloroform afforded **20** (35%, 2.36 g) as a gum: IR 3684, 3600, 1590, 1564 cm^{-1}; UV λ_{max} 277 nm (ϵ 18 300); ^1H NMR 0.08, 0.09 (2 s's, 6 H, $(CH_3)_2Si$), 0.90 (s, 9 H, t-C_4H_9Si), 1.28 (t, 6 H, J = 7.2 Hz, $(CH_3CH_2)_2N$), 2.53, 2.65 (2 m's, 2 H, 2 H-2′), 3.20 (br s, 1 H, OH), 3.85 (m, 6 H, $(CH_3CH_2)_2N$, 2 H-5′), 4.11 (m, 1 H, H-4′), 4.65 (m, 1 H, H-3′), 6.41 (t, 1 H, J = 6.4 Hz, H-1′), 7.98 (s, 1 H, H-2), 8.30 (s, 1 H, H-8). Anal. Calcd for $C_{20}H_{35}N_5O_3Si$: C, 56.98; H, 8.37; N, 16.61. Found: C, 56.65; H, 8.63; N, 16.00.

Competitive Reaction of the Magnesium Amide 18 and the Magnesium Alkoxide 21 toward Diethyl Phosphorochloridate. To a mixture of the nucleoside **17** (71.2 mg, 0.148 mmol) and **20** (62.6 mg, 0.148 mmol) in THF (2.0 mL) was added a 0.21 M solution of *tert*-butylmagnesium chloride in THF (1.41 mL, 0.296 mmol) at 25 °C. After 5 min of stirring, to this mixture was added a 0.148 M solution of diethyl phosphorochloridate (100 μL, 0.0148 mmol) at the same temperature. The reaction mixture was stirred for 5 min, quenched with saturated ammonium chloride solution (50 mL), extracted with dichloromethane (50 mL × 3), and dried. The residue of concentrated extracts was subjected to HPLC assay under aforementioned analytical conditions, which showed the product ratio of the phosphate **22** to the phosphoramidate **19** was 28.9. Use of 0.2 and 0.4 equiv of diethyl phosphorochloridate gave the **22**/**19** ratio of 26.0 and 24.0, respectively.

Competitive Reaction of the Magnesium Amide 27 and the Magnesium Alkoxide 25 toward Diethyl Phosphorochloridate. To a mixture of **23** (454.8 mg, 1.00 mmol) and **24** (7.4 mg, 0.02 mmol) in THF (5.0 mL) was added a 0.870 M solution of *tert*-butylmagnesium chloride in THF (1.17 mL, 1.02 mmol) at 20 °C. After 5 min, to this mixture was added a 0.0545 M solution of diethyl phosphorochloridate (36.5 μL, 0.0020 mmol) at the same temperature, and the mixture was stirred for 0.5 min. The reaction mixture was quenched by addition of saturated sodium bicarbonate solution (1 mL) and poured into saturated ammonium chloride solution (50 mL). The mixture was extracted with dichloromethane (50 mL × 3), dried, and concentrated to leave the residue, which was subjected to HPLC assay under the analytical conditions mentioned above. The HPLC analysis using 5′-*O*-(*tert*-butyldimethylsilyl)adenosine (t_R = 4.3 min) as an internal standard indicated that the 3′-phosphate **26** was produced in 75% yield based on the phosphorochloridate, while the probably formed phosphoroimidate **29** (<25%) was hydrolyzed to **23** during the workup.

^{31}P NMR Monitoring of Phosphorylation of 5d with Diethyl Phosphorochloridate. To a solution of **5d** (71.3 mg, 0.20 mmol) in THF (2.5 mL) were added a 0.455 M solution of *tert*-butylmagnesium chloride (0.44 mL, 0.20 mmol) in THF at 20 °C and, after 5 min of stirring, diethyl phosphorochloridate (34.0 mg, 0.20 mmol). The reaction course was monitored by examining the ^{31}P NMR spectrum at 25 °C (concentration: 0.06 M) using D_2O as an external locking solvent in a sealed capillary. The time course of product ratio of the 3′-phosphate **7d** (δ −5.9 ppm) to **28** (a regioisomeric mixture, δ −8.3, −9.3 ppm) is shown in Figure 1. After 96 h, the signals due to **28** completely disappeared, and only the signal arising from **7d** was observed.

Acknowledgment. We are indebted to Messrs. Masaaki Hirose and Hitoshi Harada for their help in the measurement of ^{31}P NMR spectra. This work was supported by the Grant-in-Aid for Scientific Research on Priority Areas No. 04226105 from the Ministry of Education, Science and Culture, Japan.

(25) Nair, V.; Richardson, S. G. *J. Org. Chem.* **1980**, *45*, 3969.