First Synthesis and Anticancer Activity of Phosmidosine and Its Related Compounds

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This paper describes the first synthesis of phosmidosine and phosmidosine B, i.e., nucleotide antibiotics composed of 8-oxoadenosine and L-proline which are connected via a unique *N*-acyl phosphoramidate linkage. Phosmidosine has a yet-undetermined chiral center at the phosphorus atom of the *N*-acyl phosphoramidate linkage. Phosmidosine B is a demethylated phosmidosine derivative with no chirality on the phosphorus. Phosmidosine B was successfully synthesized by the reaction of an *N*-acetyl-8-oxoadenosine 5′-*O*-phosphoramidite derivative with an N-protected prolinamide in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole. The successful synthesis of phosmidosine was achieved by use of a *tert*-butoxycarbonyl (Boc) group, which was found to be selectively introduced into the 7-NH function of 8-oxoadenosine and to serve as a pseudo-protecting group due to its steric effect in such manner that the unmasked 6-amino group was not phosphitylated. Final coupling reaction of the 8-oxoadenosine 5′-phosphoramidite derivative with *N*-tritylprolinamide followed by full deprotection gave a mixture of phosmidosine and its diastereoisomer. The ^{13}C NMR spectra of the diastereomers suggest that the slow-eluted diastereomer **1b** is the naturally occurring phosmidosine. The growth inhibitory activity of phosmidosine **1b**, its diastereomer **1a**, and phosmidosine B in various tumor cell lines was evaluated by the MTT assay. As the result, phosmidosine B showed high anticancer activities and both the diastereomers **1a** and **1b** of phosmidosine isolated were found to have similar but approximately 10 times higher anticancer activities than phosmidosine B. Moreover, it turned out that these phosmidosine derivatives showed characteristic inhibitory activities against cancer cells independent of their p53 phenotypes. These results suggest that phosmidosine and its related compounds would be promising as a new type of anticancer agents having a wide range of inhibitory activities against tumor cells.

Introduction

Phosmidosine (**1**) was found as a new type of antifungal antibiotic isolated from a culture filtrate of *Streptomyces durhameusis*[1] by Uramoto and Isono et al. Later, it was found by mass spectrometry and NMR spectroscopy that phosmidosine is a novel nucleotide-type antibiotic having an *N*-acyl phosphoramidate linkage which connects a nucleoside analogue, 8-oxoadenosine, with an L-proline residue.[2] They described the detailed mechanism of the action of phosmidosine showing that phosmidosine inhibits the expression of cyclin D1 synchronized with that of cyclin-dependent kinases so that the hyperphosphorylation of retinoblastoma (RB) protein is inhibited.[3] In 1996, Osada et al. reported that phosmidosine suppresses S-phase entry and arrests cell cycle progression at the G_1 phase.[4] Moreover, phosmidosine B (**2**) and C were also isolated as detransforming compounds from the fermentation broth of *Streptomyces* sp. strain RK-16 which is a producer strain of phosmidosine.[3] It turned out that phosmidosine B (**2**) shows inhibitory activity against cell cycle progression and morphological reversion activity on *src*^{ts}-NRK cells in a manner similar to that described in the case of phosmidosine (**1**).

Several natural products having *N*-acyl phosphoramidate linkages were reported (Figure 1). Agrocin 84 was discovered as an antibiotic responsible for the biological control of crown gall.[5] This natural product has two unique P−N linkages, one of which is an *N*-acyl phosphoramidate at the 5′-position. Dinogunellin, whose full structure has not been determined to date, also has this characteristic *N*-acyl phosphoramidate linkage at the 5′-position of the adenosine moiety.[6]

Several chemical properties of phosmidosine were also reported.[1] When the antibiotic was allowed to stand at room temperature in alkaline solution (pH 10) for 2 days, the inhibitory activity against spore formation of *Botryotinia fuckeliana* decreased to 60%. Heating of phosmidosine at 100 °C for 5 min resulted in a loss of 90% of the original activity at pH 10 and a loss of 60% at pH 6.

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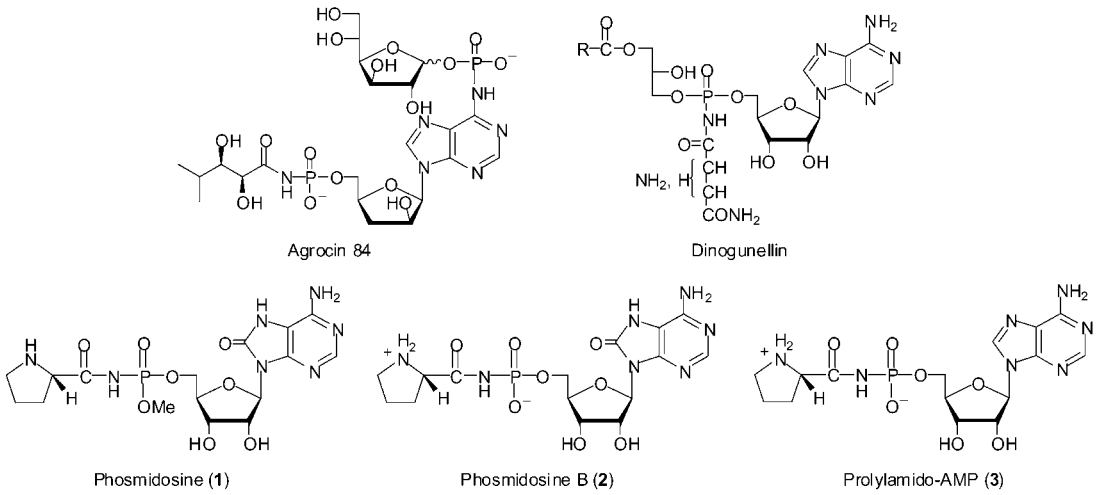

Figure 1. Natural products and synthetic materials having *N*-acyl phosphoramidate linkages.

Under acidic conditions, 20% of the activity was lost after treatment in pH 2 solution at 100 °C for 5 min. From the these results, it turned out that phosmidosine is extremely unstable under basic conditions but relatively stable under acidic conditions. Phosmidosine has a chiral center at the phosphorus atom. However, the importance of this phosphorus chiral center has yet to be clarified, especially, in respect of the structure−function relationship. In our previous paper,[7] we briefly reported the synthesis of a demethylated phosmidosine, i.e., phosmidosine B.

In this paper, we report the synthesis and anticancer activity of phosmidosine and its related compounds. A key step for the synthesis of phosmidosine is the construction of the *N*-acyl phosphoramidate linkage. We previously reported the synthesis of aminoacyl-adenylate analog[8] by the reaction of an adenosine 5′-*O*-phosphoramidite derivative with an *N*-protected amino acid amide derivative in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole[9] as an acid promoter. Therefore, we applied this original procedure to synthesize phosmidosine from 8-oxoadenosine 5′-*O*-phosphoramidite derivatives and *N*-protected prolinamide derivatives. Consequently, we found the choice of protecting groups on the adenine moiety is crucial for the successful P−N bond formation. In this paper, we also describe that both phosmidosine diastereoisomers due to the phosphorus chirality have similar biological activities.

Results and Discussion

Synthesis of Phosmidosine B. Phosmidosine B (**2**) is the demethylated form of phosmidosine at its characteristic *N*-acyl phosphoramidate linkage.[3,4] Confusion over the nomenclature of the demethylated species[3,4] of phosmidosine arose from the inconsistency between the earlier paper[4a] and the later patent.[4b] In our previous paper[7] we used the name of phosmidosine A according to the patent, but in this paper we decided to adopt phosmidosine B, the original name first reported by Osada. A similar compound **3** containing adenosine and L-proline has been synthesized by us, as mentioned before.[7] Compound **3** was found to be stable under acidic and basic conditions. Contrary to this fact, it was reported that phosmidosine is extremely unstable even under weakly basic conditions and relatively stable under acidic conditions.[1] Therefore, for the synthesis of phosmidosine B which was expected to have stability similar to that of **3**, we used a strategy similar to that used for **3**, which involves the condensation of an 8-oxoadenosine 5′-phosphoramidite derivative **9** with an *N*-protected prolinamide derivative.

The starting material compound **5** was prepared from 8-bromoadenosine by the literature previously reported.[10] The selective protection of the 5′-hydroxyl function of 6-*N*-acetyl-8-oxoadenosine (**5**) by treatment with TBDMSCl gave compound **6** in 92% yield (Scheme 1). The 2′- and 3′-hydroxyls were protected by the reaction with benzoic anhydride in the presence of 4-(dimethylamino)pyridine to give the fully protected 8-oxoadenosine derivative **7**. The 5′-OH derivative **8** was obtained in 93% yield by the selective removal of the 5′-*O*-TBDMS group by treatment with TBAF·AcOH. The 5′-OH derivative **8** was allowed to react with 2-(trimethylsilyl)ethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite[11] in the presence of diisopropylammonium tetrazolide to give the 5′-*O*-phosphoramidite derivative **9** in 86% yield.

We previously found that the solubility of the amino acid amide derivative is important for the smooth condensation of phosphoramidite derivatives with amino acid amide derivatives. Therefore, the base-labile 4,4′,4″-tris(benzoyloxy)trityl (TBTr) group[12,13] was chosen as the protecting group of the amino group of prolinamide. This base-labile protecting group can be removed together with other acyl protecting groups at the same time. Thus, *N*-TBTr prolinamide (**10**)[8] was allowed to react with the 5′-*O*-phosphoramidite derivative **9** in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole, and this *N*-acyl phosphoramidite intermediate was not purified and oxidized

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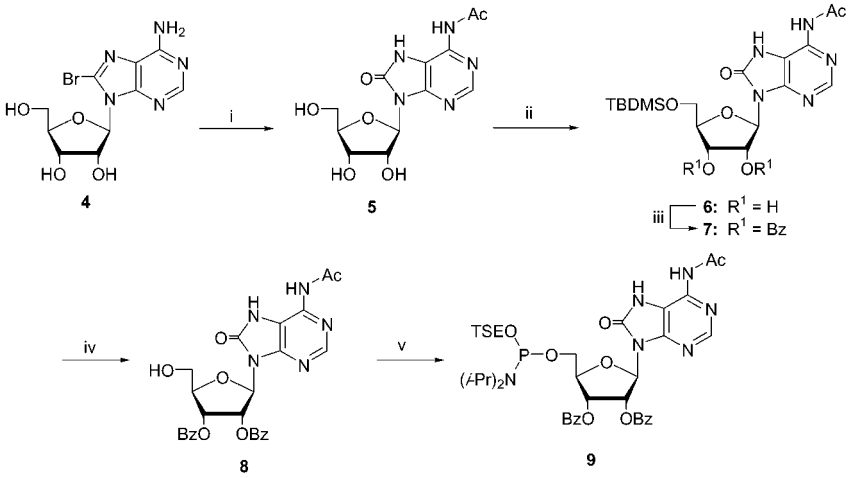
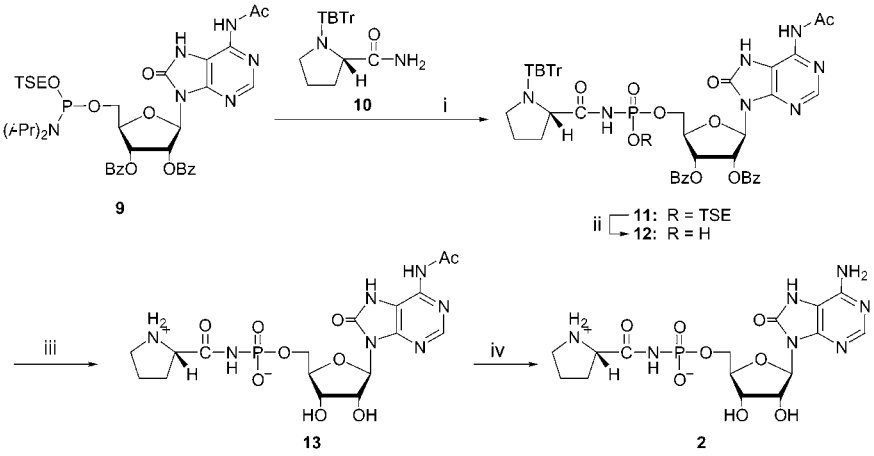
Scheme 1[a]



[a] Reagents and conditions: (i) (a) AcONa, Ac_2O, AcOH, 120 °C, 3 h, (b) c. NH_3−pyridine (3:1, v/v), rt, 6 h; (ii) TBDMSCl, pyridine, rt, 12 h; (iii) Bz_2O, DMAP, pyridine, rt, 3 h; (iv) TBAF·H_2O, AcOH, THF, rt, 12 h; (v) TSEOP(N(*i*-Pr)$_2$)$_2$, diisopropylammonium tetrazolide, CH_2Cl_2, rt, 1.5 h.

Scheme 2[a]



[a] Reagents and conditions: (i) (a) **10**, 5-(3,5-dinitrophenyl)-1*H*-tetrazole, MeCN, rt, 10 min, (b) *tert*-BuOOH, rt, 5 min; (ii) TBAF·H_2O, AcOH, THF, rt, 18 h; (iii) c. NH_3−dioxane (1:1, v/v), rt, 8 h; (iv) 1 M NaOH, 70 °C, 30 min.

by in situ treatment with *tert*-BuOOH (Scheme 2). The formation of the *N*-acyl phosphoramidate linkage was identified by ^{31}P NMR. The ^{31}P NMR spectrum of the reaction mixture showed two diastereomeric peaks at around −2 ppm, indicating the formation of an *N*-acyl phosphoramidate linkage. The coupling product **11**, thus obtained, was treated with TBAF·H_2O and AcOH to remove the 2-(trimehtylsilyl)ethyl (TSE) group to give the desired *N*-acyl phosphoramidate derivative **12** in 42% yield. It was found that the *N*-acetyl group remained after treatment of **12** with aqueous ammonia and the *N*-acetylated derivative **13** of phosmidosine B was obtained in 75% yield. Treatment of **13** with 1 M NaOH at 70 °C for 30 min followed by purification by C-18 reversed phase column chromatography gave phosmidosine B (**2**) in 53% yield. The structure of **2** was identified by NMR and MALDI-TOF mass. The ^{1}H and ^{13}C NMR chemical shifts of **2** were in good agreement with those of the literature data (Table 1).

Construction of the Methyl Ester of *N*-Acyl Phosphoramidate Linkage. Since phosmidosine is the methyl ester of phosmidosine B, the selective methylation of the *N*-acyl phosphoramidate linkage of phosmidosine B was studied by treatment with diazomethane,[14] However, all the attempts failed, because phosmidosine B exists as a zwitterion form and exhibits low solubility in organic solvents. Therefore, another synthetic plan was employed. This involves the reaction of an 8-oxoadenosine 5′-*O*-phosphoramidite derivative having a methyl group in place of the protecting group.

It was reported that phosmidosine is extremely unstable under basic conditions although phosmidosine B is stable under acidic and basic conditions. Because of this inherent instability of phosmidosine, all the protecting groups must be removed under acidic and neutral conditions. Accordingly, we searched for suitable protecting groups of the 8-oxoadenosine moiety which are compatible with the methyl ester of the *N*-acyl phosphoramidate linkage.

To see how stable the methyl ester bond of phosmidosine is under acidic conditions, an adenosine derivative **19** was chosen as the model compound of phosmidosine. The trityl group was selected as an amino protecting

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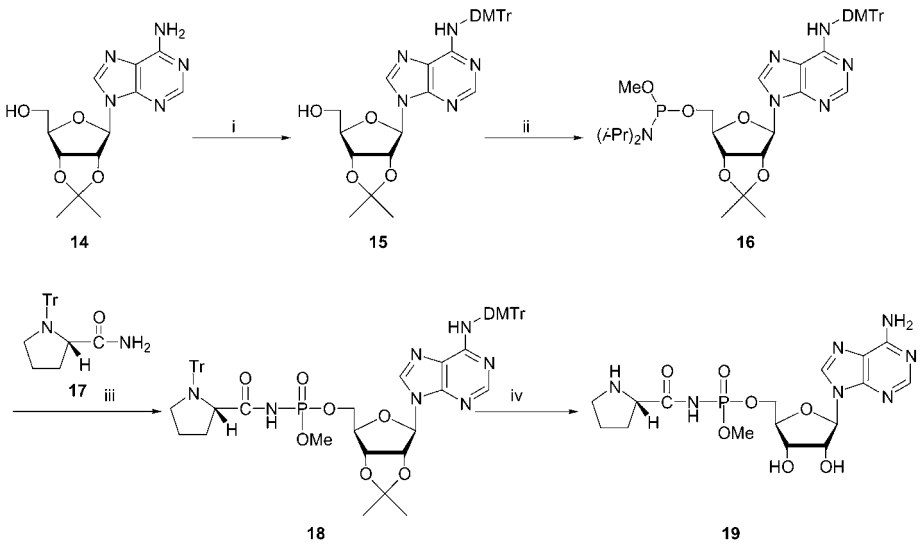
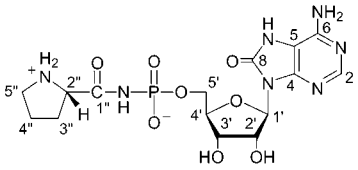
Scheme 3[a]



[a] Reagents and conditions: (i) (a) TMSCl, pyridine, rt, 30 min, (b) DMTrCl, pyridine, rt, 1 h, (c) c. NH_3−pyridine (1:4, v/v), rt, 30 min; (ii) TSEOP(N(*i*-Pr)$_2$)$_2$, diisopropylammonium tetrazolide, CH_2Cl_2, rt, 2 h; (iii) (a) **17**, 5-(3,5-dinitrophenyl)-1*H*-tetrazole, MeCN, rt, 10 min, (b) *tert*-BuOOH, MeCN, rt, 5 min, (c) I_2, pyridine−H_2O (9:1, v/v), rt, 30 min; (iv) 80% HCOOH, rt, 12 h.

Table 1. ^{1}H NMR and ^{13}C NMR Spectral Data of Phosmidosine B



	2	lit.[a,b]		**2**	lit.[a,c]
2-H	8.09 (s)	8.12 (s)	2-C	153.9	153.8
			4-C	150.2	150.0
			5-C	107.1	107.0
			6-C	149.4	149.4
			8-C	155.5	155.5
1′-H	5.85 (d, J = 5 Hz)	5.86 (d, J = 5 Hz)	1′-C	88.6	88.8
2′-H	5.11 (d, J = 5 Hz)	5.12 (d, J = 5 Hz)	2′-C	72.9	73.1
3′-H	4.53 (d, J = 5 Hz)	4.53 (d, J = 5 Hz)	3′-C	63.0	63.6
4′-H	4.07−4.18 (m)	4.18 (m)	4′-C	84.8	84.9
5′-H	4.07−4.18 (m)	4.14 (m)	5′-C	68.0	68.1
			1″-C	173.5	-
2”-H	4.36 (m)	4.10 (m)	2″-C	72.4	72.5
3”-H	2.39 (m)	2.20 (m)	3″-C	32.1	32.3
4”-H	1.89−1.98 (m)	1.99 (m)	4″-C	26.2	26.4
5”-H	3.36 (m)	3.36 (m)	5″-C	49.0	49.1

[a] Matsuura, N.; Onose, R.; Osada, H. *J. Antibiot*. **1996**, *49*, 361−365. [b] 400 MHz, D_2O. [c] 100 MHz, D_2O.

group of the amino acid amide, which is easily removed by acidic treatment and increases the solubility of the amide derivative in organic solvents. The fully protected adenosine phosphoramidite derivative **16** was synthesized as follows. Transient 5′-*O*-silylation followed by tritylation[14] of 2′,3′-*O*-isopropylideneadenosine[16] (**14**) and desilylation by treatment with aqueous NH_3 gave the 5′-OH derivative **15** in 90% yield (Scheme 3), which *in turn* was allowed to react with methyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite in the presence of diisopropylammonium tetrazolide to give the phosphoramidite derivative **16** in 85% yield. The condensation of **16** with the *N*-Tr prolinamide derivative **17** was done as described in the case of the synthesis of phosmidosine B to give the coupling product **18**. The ^{31}P NMR spectrum of the reaction mixture obtained after oxidation with *tert*-BuOOH showed several products. Two resonance signals appeared at around 10 ppm. These peaks were a pair of the diastereomeric *H*-phosphonate derivatives derived from the hydrolysis of the phosphoramidite derivative **16**. The coupling product appeared as a singlet at −0.31 ppm. These byproducts of the *H*-phosphonate derivatives could not be separated from the coupling product **18**. Therefore, this mixture was further treated with I_2 to convert these *H*-phosphonate derivatives into more polar phosphate derivatives. Thus, compound **18** was isolated in 29% yield. Deprotection of **18** with 80% formic acid at room temperature for 12 h gave the product **19** in 46% yield. The structure of **19** was confirmed by ^{1}H NMR (δ_H = 3.5 ppm, J_{HP} = 11 Hz, POMe) and ^{31}P NMR (δ_P = 12.7 ppm). The adenosine analogue of phosmidosine exhibited considerable resistance to 80% formic acid. Therefore, this synthetic strategy was ultimately applied to that of phosmidosine having an 8-oxoadenosine skeleton.

The synthesis of an 8-oxoadenosine 5′-*O*-phosphoramidite derivative **21a** was performed by the same procedure as that described in the synthesis of the adenosine derivative **16**. The 5′-OH derivative **20a** was prepared in 69% yield by the following sequential procedure: (1) the transient protection of the 5′-hydroxyl by the trimethylsilyl group, (2) *N*-dimethoxytritylation, and (3) desilylation by treatment with aqueous ammonia. The 5′-*O*-phosphitylation of **20a** in the usual way gave the phosphoramidite derivative **21a** in 63% yield (Scheme 4). The coupling reaction of **21a** with **17** in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole at room temperature for 10 min was monitored by ^{31}P NMR after oxidation of the coupling product. However, the ^{31}P NMR spectrum showed no coupling product, which should appear at around 0 ppm, was formed. Longer reaction time and use of other acid catalysts such as benzimidazolium triflate[17] resulted in the same products. It seems that the difference in reactivity between the adenosine 5′-*O*-phosphora-

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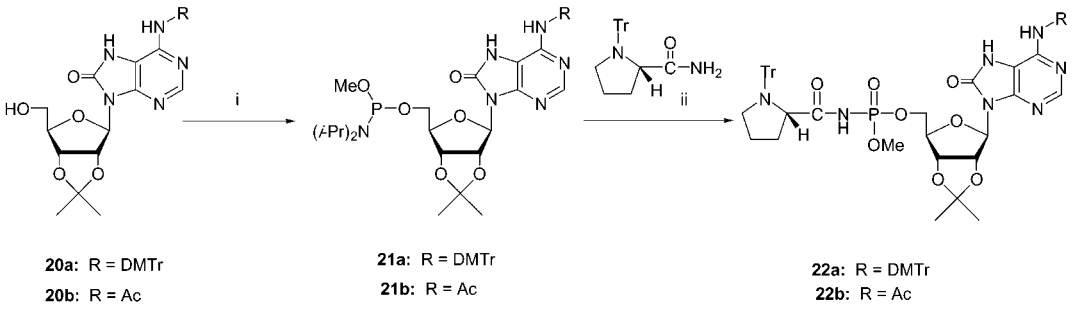
Scheme 4[a]

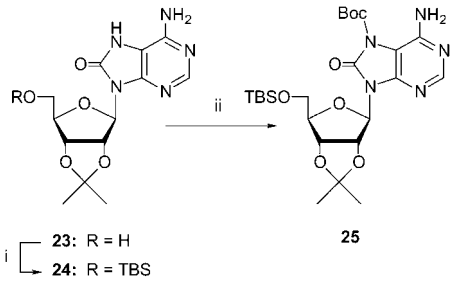


[a] Reagents: (i) TSEOP(N(*i*-Pr)$_2$)$_2$, diisopropylammonium tetrazolide, CH_2Cl_2; (ii) (a) **17**, 5-(3,5-dinitrophenyl)-1*H*-tetrazole, MeCN, (b) *tert*-BuOOH, MeCN.

midite derivative **16** and the 8-oxoadenosine counterpart **21a** is due to the difference of the orientation of their glycosidic bonds. It is well-known that the orientation of the glycosidic bond of purine bases changes from anti to syn by introduction of a large substituent to the C-8 position.[18,19] It was also reported that the glycosidic bond of 2′-deoxy-8-oxoadenosine in DNA oligomers exists in syn orientation in aqueous solution. The reason the coupling product could not be obtained seems to be due to the syn orientation of the 8-oxoadenine base and the steric hindrance of the DMTr group. Therefore, the acetyl group was introduced into the amino function in place of the DMTr group to reduce the steric hindrance of the protecting group on the base residue. The 5′-*O*-phosphoramidite derivative **21b** bearing an *N*-acetyl group was prepared in 69% yield by the 5′-*O*-phosphitylation of 6-*N*-acetyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (**20b**) which was prepared by the diol protection of **5** by the isopropylidene group. The 5′-*O*-phosphoramidite derivative **21b**, thus obtained, was found to react with **17** to give the coupling product **22b**. These results suggested that the steric hindrance of the amino protecting groups is crucial for the P−N bond formation.

Synthesis of Phosmidosine. Since the *N*-acetyl group of **22b** could not be removed under the conditions where the methyl ester of the *N*-acyl phosphoramidate linkage was stable, protecting groups for the amino function were examined. Among several protecting groups capable of removal under acidic conditions, the *tert*-butoxycarbonyl (Boc) group was found to meet the requirement of our synthetic plan. The 5′-protected derivative **24** was allowed to react with 2 equiv of di-*tert*-butyl dicarbonate in the presence of triethylamine to give highly selectively a mono *tert*-butoxycarbonylated derivative **25** (Scheme 5). The detailed ^{1}H NMR analysis of this product revealed that the Boc group was introduced into not the 6-amino group but the 7-NH function. The ^{1}H NMR spectrum of **25** showed that a new signal derived from the Boc group appeared at around 1.6 ppm with disappearance of the signal derived from 7-NH group at around 10 ppm and the original 6-amino group signal remained unchanged. Moreover, since this N^7-Boc group is expected to be capable of protection of the 6-amino group sterically and electrostatically, the 6-amino group of 8-oxoadenosine was not further protected.

Scheme 5[a]



[a] Reagents and conditions: (i) TBDMSCl, pyridine, rt, 30 min; (ii) Boc_2O, MeOH−Et_3N (9:1, v/v), rt, 30 min.

The N^7-Boc derivative **26** of 8-oxoadenosine was also synthesized in 75% yield in a more straightforward way by the reaction of 2′,3′-*O*-isopropylidene-8-oxoadenosine (**23**) with 3 equiv of di-*tert*-butyl dicarbonate (Scheme 6). The N^7 selective protection was confirmed by disappearance of the ^{1}H NMR signal of 7-NH. The 5′-*O*-phosphitylation of **26** proceeded regioselectively, and no side reaction, such as phosphitylation of the 6-amino group, was observed. Thus, the 5′-*O*-phosphoramidite derivative **27** was obtained in 70% yield. The coupling reaction of **27** with **17** was carried out in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole. After the successive oxidation with *tert*-BuOOH, the ^{31}P NMR spectrum of the reaction mixture showed the desired products were formed as evidenced by two new resonance signals at −0.40 and −0.48 ppm. The product **28** was obtained in 27% yield as a diastereomeric mixture.

Separation of the diastereoisomers failed because they have almost the same R_f values on TLC. The fully protected product **28** was treated with 80% formic acid at room temperature for 12 h to give a mixture of the desired natural product phosmidosine (**1**) and its diastereomer in 70% yield after purification using a C-18 reverse phase column. As shown in Figure 2A, preparative reverse phase HPLC resulted in successful separation of the stereoisomers of phosmidosine (**1**) which appeared at around 7 min. The fast-eluted stereoisomer **1a** and the slower-eluted one **1b** could be isolated.

The UV spectra of both isomers were observed in very similar shape with a characteristic shoulder at around 255 nm that is derived from the 8-oxoadenine of phosmidosine.[10]

The ^{31}P NMR spectra of the isolated stereoisomers **1a** and **1b** of phosmidosine showed the resonance signals at 11.89 and 12.42 ppm, respectively. There is no distinct difference in their ^{1}H NMR spectra (Table 2). However,

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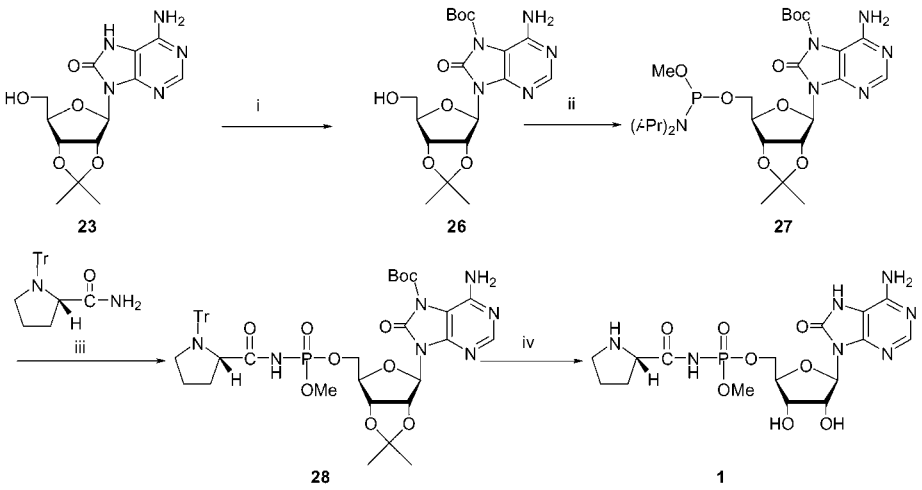
Scheme 6[a]



[a] Reagents and conditions: (i) Boc_2O, MeOH−Et_3N (9:1, v/v), rt, 1 h; (ii) $MeOP(N(i\text{-}Pr)_2)_2$, diisopropylammonium tetrazolide, CH_2Cl_2, rt, 1 h; (iii) (a) **17**, 5-(3,5-dinitrophenyl)-1*H*-tetrazole, CH_2Cl_2−MeCN (1:1, v/v), rt, 10 min, (b) *tert*-BuOOH, MeCN, rt, 5 min, (c) I_2, pyridine−H_2O (9:1, v/v), rt, 30 min; (iv) 80% HCOOH, rt, 12 h.

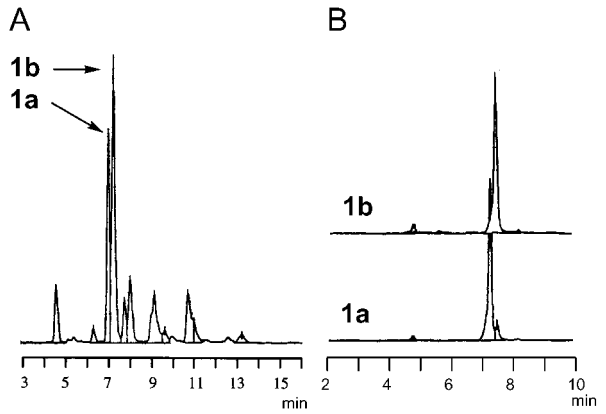
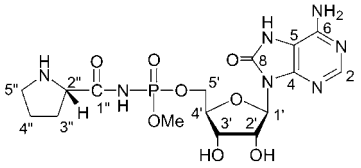


Figure 2. (A) Reverse phase HPLC profile of crude phosmidosine. (B) Reverse phase HPLC profiles of purified phosmidosine diastereoisomers

Table 2. ^{1}H NMR Spectral Data of Phosmidosine Diastereoisomers

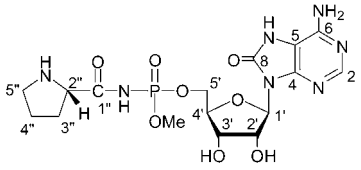


	1a	**1b**	lit.[a,b]
4″-H	1.91−2.05 (m)	1.90−2.05 (m)	1.93 (m)
3″-H	2.30−2.36 (m)	2.27−2.36 (m)	2.14 (m)
5″-H	3.26−3.41 (m)	3.26−3.50 (m)	3.31 (m)
POMe	3.55 (d, J = 11 Hz)	3.53 (d, J = 11 Hz)	3.51 (d, J = 11 Hz)
2″-H	4.10−4.22 (m)	4.08−4.21 (m)	4.07 (m)
5′-H	4.10−4.22 (m)	4.08−4.21 (m)	4.15 (m)
4′-H	4.10−4.22 (m)	4.08−4.21 (m)	4.19 (m)
3′-H	4.60 (m)	4.60 (m)	4.58 (t, J = 5 Hz)
2′-H	5.14 (dd)	5.16 (dd, J = 6 Hz)	5.15 (t, J = 5 Hz)
1′-H	5.87 (d)	5.87 (d, J = 5 Hz)	5.85 (t, J = 5 Hz)
2-H	8.09 (s)	8.09 (s)	8.30 (s)

[a] Matsuura, N.; Onose, R.; Osada, H. *J. Antibiot.* **1996**, *49*, 361−365. [b] 400 MHz, D_2O

it was found that a slight difference in ^{13}C NMR chemical shifts between the two diastereoisomers was observed, as shown in Table 3. Particularly, several differences in the chemical shifts between **1a** and **1b** were found at 3′-C, 5′-C, 1″-C, and POMe. These carbon atoms exist commonly in the vicinity of the chiral phosphorus atom. Therefore, the difference in the chemical shifts seems to reflect the chirality of the phosphorus atom. Moreover, the ^{13}C NMR data of phosmidosine reported previously are in good agreement with those of **1b**.

Table 3. ^{13}C NMR Spectral Data of Phosmidosine Diastereoisomers



	1a	**1b**	lit.[a,b]		**1a**	**1b**	lit.[a,b]
2-C	153.6	153.6	153.5	4′-C	84.3	84.4	84.4
4-C	149.8	149.8	149.8	5′-C	68.6	67.8	67.7
5-C	106.7	106.8	107.1	1″-C	177.0	178.8	179.1
6-C	149.0	149.1	149.1	2″-C	72.2	72.3	72.2
8-C	155.1	155.3	155.5	3″-C	32.2	32.4	32.2
1′-C	88.8	88.7	88.7	4″-C	26.3	26.4	26.2
2′-C	73.2	73.1	73.0	5″-C	48.9	48.8	48.7
3′-C	64.0	64.9	64.9	OMe	56.2	55.5	55.4

[a] Matsuura, N.; Onose, R.; Osada, H. *J. Antibiot.* **1996**, *49*, 361−365. [b] 100 MHz, D_2O

Anticancer Activity of Phosmidosine and Related Compounds. The aminoacyl-adenylate analogues having an adenine base and an *N*-acyl phosphoramidate linkage, which were synthesized previously by us,[8] showed weak growth inhibitory activities against KB cell. It seems that these aminoacyl-adenylate analogues act as inhibitors in a manner similar to that of phosmidosine derivatives. Therefore, the *N*-acyl phosphoramidate linkage is important in this inhibitory activity. However, no selectivity among the amino acid residues involving proline was observed in the inhibitory activity (data not shown). These results imply that the 8-oxoadenosine moiety of the phosmidosine derivatives is recognized more accurately than the amino acid residues. Table 4 represents the growth inhibitory activity of phosmidosine

Table 4. Growth Inhibitory Activity of Phosmidosine B (2) and Its *N*-acetylated Compound (13)

			IC_{50} (μM)		
cell line	origin	p53 phenotype	**2**	**13**	CDDP
KB	larynx	wild type	190.0	180.0	0.90
MKN-45	stomach	wild type	19.0	24.5	0.68
NUGC-4	stomach	wild type	8.8	15.0	0.65
MKN-28	stomach	mutant	130.0	128.0	3.68
NUGC-3	stomach	mutant	17.0	17.0	1.10
KATO III	stomach	complete deletion	28.0	29.0	2.50
SW-48	colorectal	wild type	65.0	NT[a]	0.86
LS174T	colorectal	wild type	14.8	NT[a]	0.64
SW-480	colorectal	mutant	25.0	NT[a]	2.00
HT-29	colorectal	mutant	24.0	NT[a]	6.00
SBC-3	lung	wild type	98.0	NT[a]	0.68
PC-9	lung	mutant	10.8	NT[a]	2.50

[a] Not tested.

Table 5. Growth Inhibitory Activity of Two Isomers 1a, 1b of Phosmidosine

			IC_{50} (μM)	
cell line	origin	p53 phenotype	1a	1b
KB	larynx	wild type	3.6	3.2
KATO−III	stomach	complete deletion	2.6	1.9
MKN-28	stomach	mutant	9.6	8.5
MKN-45	stomach	wild type	2.9	2.5

B and its *N*-acetyl derivative in various tumor cells. Phosmidosine B and its *N*-acetylated derivative showed the apparent growth inhibitory activities toward the tumor cell lines. These phosmidosine B derivatives showed higher inhibitory activities than the aminoacyl-adenylate analogues. Therefore, it is strongly suggested that the imidazole ring of the 8-oxoadenosine moiety is very important for the specific recognition. In general, antitumor agents, such as CDDP, showed selective inhibitory activity corresponding to the genotype of the p53 mutant. However, these phosmidosine derivatives did not show only dependence on the p53 phenotypes in their inhibitory activities.

It also seems to us that the methyl ester of the *N*-acyl phosphoramidate linkage is an important structural determinant for expression of the inhibitory activity of phosmidosine. Phosmidosine **1b** and its diasteromer **1a** showed approximately 10 times stronger antitumor activities than phosmidosine B, as shown in Tables 4 and 5. This nonionic phosphoramidate moiety is favorable for the activity of phosmidosine. It was expected that there was a difference in the growth inhibitory activity between phosmidosine two diastereomers, **1a** and **1b**. However, it turned out that both compounds have similar antitumor activities, as shown in Table 5. Moreover, inhibitory activities of both **1a** and **1b** were essentially independent of the p53 genotype of the tumor cells, as in the case of phosmidosine B.

These results suggest that the neutral structure of the methyl ester of the *N*-acyl phosphoramidate linkage and the imidazole ring structure of the 8-oxoadenosine residue are crucial for expression of the antitumor activity of phosmidosine. On the contrary, the diastereoisomers of phosmidosine shows the same antitumor activity. It was also disclosed that the chirality inversion at the phosphorus atom does not affect the inhibitory activity of phosmidosine.

Conclusion

Phosmidosine acts as an antitumor agent, which suppresses S-phase entry and arrests cell progression at the G_1 phase. Phosmidosine B was successfully synthesized by the phosphoramidite method by the reaction of the 5′-*O*-phosphoramidite derivative **9** with the *N*-TBTr prolinamide **10** in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole as a promoter. The methyl ester of the *N*-acyl phosphoramidate linkage was constructed in advance by use of the 5′-*O*-phosphoramidite derivative **27** having a methyl function. On the basis of these reactions, we realized the first synthesis of phosmidosine **1b** and its diastereomer **1a**. The MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay of these phosmidosine derivatives revealed that both diastereoisomers of phosmidosine have the p53 independent anticancer activities in tumor cell. Phosmidosine and its derivatives would be promising as a new type of novel anticancer drugs targeting cell cycle regulation. Further studies are now under way in respect to the structure−function relationship of phosmidosine.[20]

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. Triethylamine was distilled from CaH_2, after being refluxed for several hours, and stored over CaH_2. Pyridine was distilled after being refluxed over *p*-toluenesulfonyl chloride for several hours, redistilled from CaH_2, and stored over molecular sieves 4A. 2-(Trimethylsilyl)-ethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite was synthesized by the method reported previously.[11] ^{1}H NMR spectra were obtained at 270 and 400 MHz with tetramethylsilane (TMS) as an internal standard in $CDCl_3$ and with sodium (3-(trimethylsilyl)propionesulfonate (DSS) as an external standard in D_2O. ^{13}C NMR spectra were obtained at 67.8 MHz with TMS as an internal standard and with DSS as an external standard in D_2O. ^{31}P NMR spectra were obtained at 109.25 MHz using 85% H_3PO_4 as an external standard. MALDI-TOF mass spectra were obtained in the positive ion mode. The MALDI matrix used was α-cyano-4-hydroxycinnamic acid (α-CHCA, 10 mg/mL, 1:1, H_2O−MeCN, (v/v)). The calibration was performed with α-CHCA ([M + H] = 190.050) and its dimer ([M + H] = 379.093) as internal standards. ESI-TOF mass spectra were obtained in the positive ion mode. The calibration was performed with reserpine and dioctyl phthalate. Preparative HPLC was performed on a Shimadzu 6A system with a μBondapak column (Waters, C18−100 Å, 7.8 × 300 mm) using a linear gradient of 0−30% acetonitrile in 0.1 M NH_4OAc (pH 7.0) for 30 min at a flow rate of 3.0 mL/min at 50 °C. Reversed-phase column chromatography was performed using μBondasphere C18 (Waters). MTT assay of phosmidosine and its related compounds was carried out by the known method.[20]

6-*N*-Acetyl-5′-*O*-*tert*-butyldimethylsilyl-8-oxoadenosine (6). 6-*N*-Acetyl-8-oxoadenosine (**5**) (652 mg, 2 mmol) was dried by repeated coevaporation three times with dry pyridine and suspended with dry pyridine (20 mL). To this suspension was added *tert*-butyldimethylsilyl chloride (332 mg, 2.2 mmol), and the mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated to a small volume, diluted with $CHCl_3$, and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and the elution was performed with 4% MeOH/$CHCl_3$. The fractions containing **6** were combined and concentrated under reduced pressure to give **6** (813 mg, 92%) as a colorless foam: ^{1}H NMR (C_5D_5N) δ 0.07 (6H, s), 0.88 (9H, s), 2.26 (3H, s), 4.15−4.22 (1H, m), 4.30−4.36 (1H, m), 4.59−4.65 (1H, m), 5.14 (1H, m), 5.82 (1H, m), 6.83 (1H, d, J = 4.6 Hz), 8.57 (1H, s), 11.07 (1H, bs), 12.07 (1H, bs); ^{13}C NMR ($CDCl_3$) δ −5.42, −5.33, 18.00, 23.11, 25.77,

(20) Carmichael.; DeGraff, W. G.; Gazar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936−942.

63.24, 69.80, 69.99, 84.12, 85.84, 111.00, 137.99, 149.72, 150.33, 151.12, 169.36. Anal. Calcd for $C_{18}H_{29}N_5O_6 \cdot 1/2H_2O$: C, 48.20; H, 6.74; N, 15.61. Found: C, 47.89; H, 6.46; N, 15.64.

6-*N*-Acetyl-2′,3′-di-*O*-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-8-oxoadenosine (7). 6-*N*-Acetyl-5′-*O*-*tert*-butyldimethylsilyl-8-oxoadenosine (**6**) (440 mg, 1 mmol) was dried by repeated coevaporation three times with dry pyridine and dissolved in dry pyridine (10 mL). To this solution were added benzoic anhydride (543 mg, 2.4 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (2.4 mg, 0.02 mmol), and the mixture was stirred under argon atmosphere at room temperature for 3 h. The mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was applied to a silica gel column, and the elution was performed with hexane−ethyl acetate (7:3, v/v). The fractions containing **7** were combined and concentrated under reduced pressure to give **7** (567 mg, 87%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.86 (9H, s), 2.27 (3H, s), 3.97−4.11 (2H, m), 4.46 (1H, m), 6.12 (1H, dd, J = 5.9 Hz, J = 5.9 Hz), 6.31 (1H, d, J = 4.6 Hz), 6.66 (1H, dd, J = 4.6 Hz, J = 5.9 Hz), 8.31 (1H, s), 8.43 (1H, bs), 9.41 (1H, bs); ^{13}C NMR ($CDCl_3$) δ −5.42, 18.35, 23.92, 25.84, 63.11, 71.52, 72.71, 82.41, 84.64, 108.45, 128.36, 128.41, 128.54, 128.81, 129.20, 129.76, 133.35, 133.53, 137.48, 146.54, 150.57, 150.66, 150.76, 165.37, 165.62, 169.94. Anal. Calcd for $C_{32}H_{37}N_5O_8$: C, 59.33; H, 5.76; N, 10.81. Found: C, 59.60; H, 5.63; N, 10.82.

6-*N*-Acetyl-2′,3′-di-*O*-benzoyl-8-oxoadenosine (8). 6-*N*-Acetyl-2′,3′-di-*O*-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-8-oxoadenosine (**7**) (7.54 g, 11.6 mmol) was dissolved in dry THF (120 mL). To this solution were added tetrabutylammonium fluoride monohydrate (4.57 g, 17.5 mmol) and AcOH (1.0 mL, 17.5 mmol). After being stirred at room temperature for 7 h, the mixture was concentrated to a small volume, diluted with $CHCl_3$, and washed three times with 0.5 M triethylammonium hydrogen carbonate. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was applied to a silica gel column, and the elution was performed with $CHCl_3$−MeOH (100:1.25, v/v). The fractions containing **8** were combined and concentrated under reduced pressure to give **8** (6.21 g, 93%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 2.28 (3H, s), 3.94−4.06 (2H, m), 4.56 (1H, s), 5.68−5.72 (1H, m), 6.03−6.06 (1H, m), 6.39−6.43 (1H, dd, J = 5.3 Hz, J = 7.9 Hz), 6.57 (1H, d), 7.15−7.72 (6H, m), 7.86 (2H, d, J = 8.6 Hz), 8.07 (2H, d, J = 8.6 Hz), 8.38 (1H, s), 9.41 (1H, bs), 9.55 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 23.90, 62.86, 71.56, 73.26, 84.60, 85.51, 109.02, 128.37, 128.46, 128.55, 129.13, 129.76, 133.55, 133.60, 138.17, 150.17, 150.24, 150.55, 165.32, 165.59, 169.92. Anal. Calcd for $C_{26}H_{23}N_5O_8$: C, 56.62; H, 4.57; N, 12.69. Found: C, 56.58; H, 4.44; N, 12.38.

6-*N*-Acetyl-2′,3′-di-*O*-benzoyl-8-oxoadenosine 5′-[2-(Trimethylsilyl)ethyl *N*,*N*-Diisopropylphosphoramidite] (9). 6-*N*-Acetyl-2′,3′-di-*O*-benzoyl-8-oxoadenosine (**8**) (2.67 g, 5 mmol) was dried by repeated coevaporation three times with dry pyridine twice with dry toluene, and dissolved in dry CH_2Cl_2 (50 mL). To this solution were added 2-(trimethylsilyl)ethyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (2.27 g, 6.5 mmol) and diisopropylammonium tetrazolide (428 mg, 2.5 mmol). After being stirred under argon atmosphere at room temperature for 1.5 h, the mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was applied to a silica gel column, and the elution was performed with hexane−ethyl acetate (6:4, v/v) containing 1% triethylamine. The fractions containing **9** were combined and concentrated under reduced pressure to give **9** (3.37 g, 86%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 146.38, 146.41; ^{1}H NMR ($CDCl_3$) δ −0.04 (9H, s), 0.91−0.97 (2H, m), 1.01−1.14 (12H, m), 2.28 (3H, s), 3.51−3.75 (4H, m), 3.91−4.12 (2H, m), 4.60 (1H, m), 6.13−6.17 (1H, m), 6.30 (1H, d, J = 4.3 Hz), 6.66−6.69 (1H, m), 7.24−7.57 (6H, m), 7.85 (2H, d, J = 8.3 Hz), 8.00 (2H, d, J = 7.9 Hz), 8.35 (1H, s); ^{13}C NMR ($CDCl_3$) δ −1.51, −1.13, 19.91, 20.00, 23.78, 24.40, 24.51, 42.61, 42.64, 42.79, 42.82, 60.88, 61.15, 62.95, 63.06, 63.18, 63.29, 72.04, 73.14, 73.23, 81.31, 81.42, 84.78, 108.21, 108.25, 128.10, 128.30, 128.57, 128.70, 128.91, 129.09, 129.15, 129.70, 133.28, 133.51, 137.66, 150.33, 150.48, 150.80, 165.25, 165.68, 165.71, 170.33. Anal. Calcd for $C_{37}H_{49}N_6O_9PSi$: C, 56.91; H, 6.32; N, 10.76. Found: C, 56.67; H, 6.10; N, 10.61.

Triethylammonium 6-*N*-Acetyl-2′,3′-di-*O*-benzoyl-8-oxoadenosine 5′-[*N*-{*N*-(4,4′,4″-Trisbenzoyloxytrityl)-L-prolyl}phosphoramidate] (12). A mixture of *N*-TBTr-L-prolinamide (**10**) (717 mg, 1.0 mmol) and phosphoramidite **9** (1.17 g, 1.5 mmol) was dried by repeated coevaporation three times each with dry pyridine and with dry toluene and finally dissolved in dry MeCN (15 mL). The solution was added to 5-(3,5-dinitrophenyl)-1*H*-tetrazole (709 mg, 3.0 mmol), which was dried by repeated coevaporation with dry pyridine and dry toluene. The mixture was stirred at room temperature for 10 min. To the solution was added *tert*-butyl hydroperoxide (623 μL, 5.0 mmol). After being stirred at room temperature for 5 min, the mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was dissolved in dry THF (45 mL), and tetrabutylammonium fluoride monohydrate (1.18 g, 4.5 mmol) and AcOH (258 μL, 4.5 mmol) were added to this mixture. After being stirred at room temperature for 8 h, the mixture was concentrated under reduced pressure to a small volume, diluted with $CHCl_3$, and washed three times with 0.5 M triethylammonium hydrogen carbonate. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with $CHCl_3$−MeOH (100:2, v/v) containing 1% triethylamine. The fractions containing **12** were combined and concentrated to give **12** (587 mg, 42%) as a yellow foam: ^{31}P NMR ($CDCl_3$) δ −6.8; ^{1}H NMR ($CDCl_3$) δ 0.87 (2H, m), 1.24 (9H, t, J = 7.3 Hz), 1.41−1.66 (2H, m), 2.33 (3H, s), 3.05 (6H, m), 3.28 (1H, m), 3.80 (1H, m), 4.36−4.63 (3H, m), 6.15 (1H, m), 6.31 (1H, d, J = 4.3 Hz), 6.59 (1H, m), 7.15 (6H, d, J = 8.5 Hz), 7.21−7.42 (26H, m), 7.77−7.91 (4H, m), 8.38 (1H, s), 11.55 (1H, s).

6-*N*-Acetyl-8-oxoadenosine 5′-(*N*-L-Prolylphosphoramidate) (13). Triethylammonium 6-*N*-acetyl-2′,3′-di-*O*-benzoyl-8-oxoadenosine 5′-[*N*-{*N*-(4,4′,4″-trisbenzoyloxytrityl)-L-prolyl}phosphoramidate] (**12**) (977 mg, 0.69 mmol) was dissolved in concentrated NH_3−dioxane (7 mL, (1:1, v/v)), and this solution was stirred at room temperature for 8 h. This mixture was diluted to a small volume under reduced pressure, diluted with H_2O, and washed five times with ether. The aqueous layer was concentrated under reduced pressure. The residue was applied to a C-18 reversed-phase silica gel column, and elution was performed with H_2O−MeCN (98:2, v/v). The fraction containing **13** were combined and lyophilized to give **13** (258 mg, 75%) as a colorless powder: ^{31}P NMR (D_2O) δ −5.40; ^{1}H NMR (D_2O) δ 1.97−1.99 (3H, m), 2.28 (3H, s), 2.41 (1H, m), 3.34 (2H, m), 4.13−4.21 (3H, m), 4.37 (1H, m), 4.55 (1H, dd, J = 5.0 Hz), 5.16 (1H, dd, J = 5.0 Hz), 5.85 (1H, d, J = 4.6 Hz), 8.44 (1H, s). MALDI-TOF mass m/z Calcd for $C_{17}H_{25}N_7O_9P$ 502.15; Observed [M + H] 502.15.

8-Oxoadenosine 5′-(*N*-L-Prolylphosphoramidate) (Phosmidosine B) (2). 6-*N*-Acetyl-8-oxoadenosine 5′-(*N*-L-prolylphosphoramidate) (**13**) (30 mg, 0.06 mmol) was dissolved in 1 M NaOH, and this solution was stirred at 70 °C for 30 min. After cooling to room temperature, the reaction mixture was neutralized by addition of Dowex 50W×8 (pyridinium form). The resin was filtered off and washed with H_2O. The filtrate was concentrated and coevaporated several times with H_2O under reduced pressure. The residue was applied to a C-18 reversed-phase silica gel column, and elution was performed with H_2O−MeCN (98:2, v/v). The fraction containing **2** were combined and lyophilized to give **2** (14.5 mg, 53%) as a colorless powder: ^{31}P NMR (D_2O) δ −5.13; ^{1}H NMR (D_2O) δ 1.89−1.98 (3H, m), 2.39 (1H, m), 3.36 (2H, m), 4.07−4.18 (3H, m), 4.36 (1H, m), 4.53 (1H, dd, J = 5.3 Hz), 5.11 (1H, dd, J = 5.0 Hz), 5.85 (1H, d, J = 4.9 Hz), 8.09 (1H, s); ^{13}C NMR (D_2O) δ 26.20, 32.09, 49.00, 63.02 (d, J = 13.4 Hz), 67.98 (d, J = 4.9 Hz), 72.38, 72.92, 84.84 (d, J = 8.5 Hz), 88.62, 122.07, 149.38,

150.15, 153.94, 155.51, 173.46. MALDI-TOF mass *m*/*z* Calcd for $C_{15}H_{23}N_7O_8P$ 460.13; Observed [M + H] 460.15.

6-*N*-(4,4′-Dimethoxytrityl)-2′,3′-*O*-isopropylideneadenosine (15). 2′,3′-*O*-Isopropylideneadenosine (**14**) (307 mg, 1 mmol) was dried by repeated coevaporation three times with dry pyridine and finally dissolved in dry pyridine (8 mL). To this solution was added chlorotrimethylsilane (190 μL, 1.5 mmol). After being stirred at room temperature for 30 min, the mixture was allowed to react with 4,4′-dimethoxytrityl chloride (407 mg, 1.2 mmol). After stirred at room temperature for 1 h, concentrated NH_3 (2 mL) was added to this solution, and stirring was continued at room temperature for an additional 30 min. The mixture was concentrated to a small volume under reduced pressure. The residue was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (6:4−4:6, v/v) containing 1% pyridine. The fractions containing **15** were combined and concentrated to give **15** (548 mg, 90%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 1.36 (3H, s), 1.64 (3H, s), 3.71−3.96 (8H, m), 4.53 (1H, m), 5.08 (1H, m), 5.18 (1H, m, J = 5.6 Hz), 5.83 (1H, d, J = 5.0 Hz), 6.79 (4H, d, J = 8.9 Hz), 6.98 (1H, s), 7.16−7.33 (9H, m), 7.78 (1H, s), 8.00 (1H, s); ^{13}C NMR ($CDCl_3$) δ 21.41, 25.18, 27.60, 55.08, 63.23, 70.67, 81.53, 82.87, 85.94, 94.11, 112.98, 113.72, 122.13, 125.07, 126.65, 127.67, 127.99, 128.52, 128.79, 129.85, 136.94, 136.96, 137.56, 139.32, 144.93, 146.96, 151.57, 154.34, 158.07. Anal. Calcd for $C_{34}H_{35}N_5O_6 \cdot 8/5H_2O$: C, 63.96; H, 6.03; N, 10.97. Found: C, 64.38; H, 5.55; N, 10.58. MALDI-TOF mass *m*/*z* Calcd for $C_{34}H_{36}N_5O_6$ 610.27; Observed [M + H] 610.27.

6-*N*-(4,4′-Dimethoxytrityl)-2′,3′-*O*-isopropylideneadenosine 5′-(Methyl *N*,*N*-Diisopropylphosphoramidite) (16). 6-*N*-(4,4′-Dimethoxytrityl)-2′,3′-*O*-isopropylideneadenosine (**15**) (2.26 g, 3.71 mmol) and diisopropylammonium tetrazolide (381 mg, 2.23 mmol) were dried by repeated coevaporation three times each with dry pyridine and with dry toluene and finally dissolved in dry CH_2Cl_2 (40 mL). To this solution was added methyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (1.18 mL, 4.45 mmol). After being stirred under argon atmosphere at room temperature for 2 h, the mixture was diluted with $CHCl_3$, and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (8:2, v/v) containing 1% pyridine. The fractions containing **16** were combined and concentrated to give **16** (2.43 g, 85%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 149.95, 149.98; ^{1}H NMR (DMSO-d_6) δ 1.03−1.30 (12H, m), 1.37 (3H, s), 1.61 (3H, s), 3.34−3.36 (3H, m), 3.39−3.76 (4H, m), 4.46−4.50 (1H, m), 4.98−5.01 (1H, m), 5.26−5.31 (1H, m), 6.12−6.15 (1H, m), 6.76−6.79 (4H, m), 6.80 (1H, s), 7.16−7.35 (9H, m), 8.01 (1H, s), 8.07 (1H, s).

6-*N*-(4,4′-Dimethoxytrityl)-2′,3′-*O*-isopropylideneadenosine 5′-[Methyl *N*-(*N*-Trityl-L-prolyl)phosphoramidate] (18). A mixture of *N*-Tr-L-prolinamide (**17**) (357 mg, 1.0 mmol) and phosphoramidite **16** (1.16 g, 1.5 mmol) were dried by repeated coevaporation three times each with dry pyridine and with dry toluene and finally dissolved in dry MeCN (15 mL). The solution was added to 5-(3,5-dinitrophenyl)-1*H*-tetrazole (709 mg, 3.0 mmol), which was dried by repeated coevaporation with dry pyridine and dry toluene. The mixture was stirred at room temperature for 10 min. To the mixture was added *tert*-butyl hydroperoxide (623 μL, 5.0 mmol). After the mixture was stirred at room temperature for 5 min, a 2 M solution of I_2 in pyridine−H_2O (9:1, v/v) was added to the mixture. After being stirred at room temperature for 30 min, the mixture was diluted with $CHCl_3$ and washed three times with sat. sodium thiosulfate. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexanes−ethyl acetate (40:60−35:65, v/v). The fractions containing **18** were combined and concentrated to give **18** (304 mg, 29%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ −0.31; ^{1}H NMR (DMSO-d_6) δ 0.89−1.30 (4H, m), 1.33,1.37 (3H, 2s), 1.59, 1.62 (3H, 2s), 2.97−3.07 (1H, m), 3.26−3.31 (1H, m), 3.76−3.85 (9H, m), 3.95 (1H, m), 4.47−4.52 (2H, m), 5.07−5.14 (1H, m), 5.28−5.31 (1H, m), 6.12−6.13 (1H, m), 6.75−6.85 (4H, m), 6.92 (1H, m), 7.08−7.45 (24H, m); ^{13}C NMR ($CDCl_3$) δ 24.28, 24.34, 25.32, 25.35, 27.19, 27.21, 31.76, 50.74, 54.27, 54.31, 54.35, 54.40, 55.16, 65.57, 65.69, 67.04, 67.13, 70.55, 70.57, 70.72, 78.22, 78.26, 80.88, 80.94, 84.01, 84.10, 84.40, 84.51, 89.93, 90.07, 100.40, 112.99, 113.04, 113.79, 114.54, 114.69, 121.10, 126.51, 126.60, 126.72, 127.68, 127.76, 127.84, 128.04, 128.60, 128.63, 128.85, 129.01, 129.06, 129.90, 129.93, 137.20, 137.24, 37.26, 138.69, 143.81, 145.17, 145.22, 148.10, 148.17, 152.32, 153.93, 153.94, 158.04, 158.12, 177.57, 177.62.

Adenosine 5′-(Methyl *N*-Prolylphosphoramidate) (19). *O*-[6-*N*-(4,4′-Dimethoxytrityl)]-2′,3′-*O*-isopropylideneadenosine-5′-*O*-yl)-*O*′-methyl-*N*-(*N*-trityl-L-prolyl)phosphoramidate (**18**) (104 mg, 0.1 mmol) was dissolved in 80% formic acid (1.0 mL). After being stirred at room temperature for 12 h, the mixture was diluted with $CHCl_3$ and washed three times with $CHCl_3$. The aqueous layer was concentrated and coevaporated three times with H_2O to remove the last traces of formic acid. The residue was applied to a C-18 reversed-phase silica gel column, and elution was performed with CH_3CN-H_2O (98:2−98:4, v/v). The fraction containing **19** were combined and lyophilized to give **19** (23 mg, 46%) as a colorless powder: ^{31}P NMR ($CDCl_3$) δ 12.7; ^{1}H NMR ($CDCl_3$) δ 1.90−2.03 (3H, m), 2.36 (1H, m), 3.56 H, d, J = 11.2 Hz), 4.12−4.19 (3H, m), 4.35 (1H, bs), 4.50 (1H, m), 6.07 (1H, d), 8.13 (1H, s), 8.38 (1H, s). ESI-mass *m*/*z* Calcd for $C_{16}H_{25}N_7O_7P$, 458.2; Observed 458.2 (M + H).

6-*N*-[(4,4′-Dimethoxytrityl)]-2′,3′-*O*-isopropylidene-8-oxoadenosine (20a). 2′,3′-*O*-Isopropylidene-8-oxoadenosine (1.29 g, 4 mmol) was dried by repeated coevaporation three times with dry pyridine and finally dissolved in dry pyridine (32 mL). To this solution was added chlorotrimethylsilane (762 μL, 6.0 mmol). After being stirred at room temperature for 1 h, 4,4′-dimethoxytrityl chloride (1.68 g, 5.2 mmol) was added to the mixture. After the mixture was stirred at room temperature for 1 h, concentrated NH_3 (8 mL) was added to the solution. After being stirred at room temperature for 30 min, the mixture was concentrated under reduced pressure. The residue was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (6:4−4:6, v/v) containing 1% pyridine. The fractions containing **20a** were combined and concentrated to give **20a** (1.73 g, 69%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 1.27 (3H, s), 1.48 (3H, s), 3.37−3.56 (2H, m), 3.70 (6H, s), 4.01 (1H, m, J = 3.1 Hz), 4.82−4.92 (2H, m, J = 3.1 Hz), 5.36 (1H, dd, J = 6.4 Hz), 5.81 (1H, d, J = 2.6 Hz), 6.82 (4H, d, J = 8.9 Hz), 7.13−7.31 (9H, m), 7.70 (1H, s), 8.55 (1H, bs), 10.94 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 25,49, 27.68, 27.99, 63.41, 81.19, 81.26, 85.16, 87.00, 89.11, 102.08, 110.94, 147.12, 147.87, 149.04, 149.76, 153.07.

6-*N*-(4,4′-Dimethoxytrityl)-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-(Methyl *N*,*N*-Diisopropylphosphoramidite) (21a). 6-*N*-[(4,4′-Dimethoxytrityl)]-2′,3′-*O*-isopropylidene-8-oxoadenosine (**20a**) (1.25 g, 2.0 mmol) and diisopropylammonium tetrazolide (223 mg, 1.3 mmol) were dried repeated coevaporation three times each with dry pyridine and with dry toluene and finally dissolved in dry CH_2Cl_2 (20 mL). To this solution was added methyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (746 μL, 2.6 mmol), and stirring was continued under argon atmosphere at room temperature 1 h. The mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (1:1, v/v) containing 1% triethylamine. The fractions containing **21a** were combined and concentrated to give **21a** (990 mg, 63%) as a colorless foam: ^{31}P NMR ($CDCl_3$)

δ 149.12, 149.42; ^{1}H NMR (DMSO-d_6) δ 0.82 (3H, d), 0.97−1.06 (9H, m), 1.25 (3H, s), 1.45 (3H, s), 3.15−3.22 (4H, m), 3.40 (3H, m), 4.10 (1H, m), 4.86 (1H, m), 5.36 (1H, dd, $J_{2',3'} = 8.9$ Hz), 5.82 (1H, d, $J_{1',2'} = 2.3$ Hz), 8.18 (1H, 2s); ^{13}C NMR ($CDCl_3$) δ 22.86, 22.94, 22.97, 24.42, 24.48, 24.52, 24.59, 24.65, 24.68, 24.76, 24.78, 25.45, 25.50, 27.22, 42.57, 42.61, 42.76, 42.79, 45.26, 45.35, 50.53, 50.79, 55.13, 62.99, 63.14, 63.24, 63.38, 70.32, 77.47, 82.22, 82.40, 85.85, 85.96, 86.08, 86.19, 86.73, 86.81, 113.26, 113.57, 127.02, 127.93, 128.42, 129.76, 136.99, 145.35, 145.88, 147.24, 150.38, 151.79, 158.31. Anal.Calcd for $C_{41}H_{51}N_6O_8P\cdot H_2O$: C, 61.18; H, 6.64; N, 10.44. Found: C, 60.71; H, 6.72; N, 10.21.

6-*N*-Acetyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (20b). 6-*N*-Acetyl-8-oxoadenosine (**6**) (651 mg, 2.0 mmol) was suspended in dry acetone (20 mL), and to this suspension were added acetone dimethylacetal (4.92 mL, 40 mmol) and *p*-toluenesulfonic acid monohydrate (689 mg, 4 mmol). After being stirred at room temperature for 30 min, the mixture was neutralized by addition of 5% $NaHCO_3$ (20 mL) at 0 °C and warmed to room temperature. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was diluted with $CHCl_3$−isopropyl alcohol (3:1, v/v) and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure to give **20b** (730 mg, 100%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.30 (3H, s), 1.51 (3H, s), 2.13 (3H, s), 3.47−3.50 (2H, m), 4.05 (1H, m), 4.90 (1H, m, $J = 3.3$ Hz), 5.45 (1H, dd, $J = 6.1$ Hz), 5.94 (1H, d, $J = 2.3$ Hz), 8.40 (1H, s), 10.33 (1H, bs), 10.84 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 23.14, 25.26, 27.11, 61.61, 81.63, 86.20, 87.10, 110.92, 112.75, 138.00, 149.62, 150.56, 169.18. MALDI-TOF mass *m*/*z* Calcd for $C_{15}H_{20}N_5O_6$ 366.14; Observed [M + H] 366.16.

6-*N*-Acetyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-(Methyl *N*,*N*-Diisopropylphosphoramidite) (21b). A mixture of 6-*N*-Acetyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (**20b**) (365 mg, 1.0 mmol) and diisopropylammonium tetrazolide (129 mg, 0.75 mmol) were dried repeated coevaporation three times each with dry pyridine and with dry toluene and finally dissolved in dry CH_2Cl_2 (10 mL). To this solution was added methyl *N*,*N*,*N*′,*N*′-tetraisopropylphosphorodiamidite (430 μL, 1.5 mmol). After being stirred under argon atmosphere at room temperature 1 h, the mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (70:30−0:100, v/v) containing 1% triethylamine. The fractions containing **21b** were combined and concentrated to give **21b** (362 mg, 69%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 149.27, 149.51; ^{1}H NMR ($CDCl_3$) δ 1.09−1.17 (12H, m), 1.37 (3H, s), 1.59 (3H, s), 2.13 (3H, s), 3.34−3.41 (3H, m), 3.51−3.61 (2H, m), 3.74−3.89 (2H, m), 4.36 (1H, m), 5.02−5.06 (1H, m), 5.52−5.58 (1H, m), 6.24 (1H, d $J = 2.0$ Hz), 8.34 (1H, 2s), 8.44 (1H, bs), 9.34 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 24.06, 24.38, 24.52, 24.60, 24.63, 24.70, 24.80, 25.55, 25.57, 27.26, 42.63, 42.66, 42.81, 42.84, 48.55, 50.57, 50.60, 50.83, 50.86, 63.08, 63.22, 63.33, 63.47, 82.32, 82.35, 82.45, 86.47, 86.58, 86.68, 86.92, 86.98, 100.21, 108.64, 08.65, 113.75, 113.81, 137.34, 150.43, 150.53, 150.76, 150.82, 169.51, 169.54.

2′,3′-*O*-Isopropylidene-8-oxoadenosine (23). 8-Oxoadenosine[21] (623 mg, 2.2 mmol) was suspended to dry acetone (22 mL). To this suspension were added acetone dimethylacetal (5.4 mL, 44 mmol) and *p*-toluenesulfonic acid monohydrate (758 mg, 4.4 mmol). After being stirred at room temperature for 30 min, the mixture was neutralized by addition of sat. $NaHCO_3$ (22 mL) at 0 °C and warmed to room temperature. The precipitate was filtered off, and the filtrate was concentrated under reduced pressure. The residue was diluted with $CHCl_3$−isopropyl alcohol (3:1, v/v) and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure to give **23** (649 mg, 91%) as a white solid: ^{1}H NMR (DMSO-d_6) δ 1.29 (3H, s), 1.49 (3H, s), 3.36−3.53 (2H, m), 4.06 (1H, m), 4.86−4.96 (2H, m, $J = 3.3$ Hz), 5.39 (1H, dd, $J = 6.3$ Hz), 5.84 (1H, d, $J = 2.3$ Hz), 6.54 (2H, bs), 8.03 (1H, s), 10.40 (1H, bs); ^{13}C NMR (DMSO-d_6) δ 25.25, 27.15, 61.74, 79.09, 81.54, 81.65, 86.38, 86.56, 103.43, 112.70, 145.97, 147.00, 150.71, 150.87. MALDI-TOF mass *m*/*z* Calcd for $C_{13}H_{18}N_5O_5$ 324.13; Observed [M + H] 324.12.

5′-*O*-*tert*-Butyldimethylsilyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (24). 2′,3′-*O*-Isopropylidene-8-oxoadenosine (326 mg, 1 mmol) was dried by repeated coevaporation three times with dry pyridine and finally dissolved in dry pyridine (10 mL). To this solution was added *tert*-butyldimethylchlorosilane (196 mg, 1.3 mmol). After being stirred at room temperature for 30 min, the mixture was concentrated under reduced pressure. The residue was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with $CHCl_3$−MeOH (100:2.5, v/v). The fractions containing **24** were combined and concentrated to give **24** (377 mg, 86%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 0.04 (6H, s), 0.84 (9H, s), 1.37 (3H, s), 1.58 (3H, s), 3.82−3.92 (1H, dd, $J = 5.1$ Hz), 3.97−4.04 (1H, m), 4.28 (1H, m, $J = 5.1$ Hz), 4.93 (1H, dd, $J = 3.3$ Hz), 5.52 (1H, dd, $J = 6.3$ Hz), 6.15 (1H, d, $J = 1.6$ Hz), 8.04 (1H, s), 10.02 (1H, bs); ^{13}C NMR ($CDCl_3$) δ −5.14, −5.05, 18.46, 25.45, 25.93, 27.20, 63.86, 81.84, 82.73, 87.25, 88.28, 103.83, 113.68, 146.62, 146.80, 151.58, 152.23. MALDI-TOF mass *m*/*z* Calcd for $C_{19}H_{32}N_5O_5Si$ 438.22; Observed [M + H] 438.22.

***N*^{7}-*tert*-Butoxycarbonyl-5′-*O*-*tert*-butyldimethylsilyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (25).** 5′-*O*-*tert*-Butyldimethylsilyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (**24**) (88 mg, 0.2 mmol) was dissolved in MeOH−triethylamine (20 mL, (9:1, v/v)). To this solution was added di-*tert*-butyl-dicarbonate (92 μL, 0.4 mmol). After being stirred at room temperature for 30 min, the mixture was diluted with $CHCl_3$ and washed three times with H_2O. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (9:1−8:2, v/v). The fractions containing **25** were combined and concentrated to give **25** (102 mg, 94%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 0.00 (6H, s), 0.85 (9H, s), 1.34 (3H, s), 1.53 (3H, s), 1.60 (9H, s), 3.69−3.78 (2H, m), 4.11 (1H, m), 4.92 (1H, m, $J_{3',4'} = 3.3$ Hz), 5.52 (1H, dd, $J = 2.0$ Hz, $J = 6.3$ Hz), 5.99 (1H, d, $J_{1',2'} = 1.6$ Hz), 7.08 (2H, bs), 8.17 (1H, s); ^{13}C NMR ($CDCl_3$) δ −5.22, −5.17, 18.51, 25.68, 26.01, 27.30, 28.01, 63.33, 81.93, 82.28, 86.63, 87.15, 87.38, 101.95, 113.68, 147.87, 147.96, 148.68, 149.87, 153.60. MALDI-TOF mass *m*/*z* Calcd for $C_{24}H_{40}N_5O_7Si$ 538.27; Observed [M + H] 538.04.

***N*^{7}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (26).** 2′,3′-*O*-Isopropylidene-8-oxoadenosine (**23**) (485 mg, 1.5 mmol) was dissolved in MeOH−triethylamine (15 mL, (9:1, v/v)). To this solution was added di-*tert*-butyl dicarbonate (1.03 mL, 4.5 mmol). After being stirred at room temperature for 1 h, the mixture was diluted with $CHCl_3$ and washed three times with H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (7:3, v/v). The fractions containing **26** were combined and concentrated to give **26** (478 mg, 75%) as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 1.29 (3H, s), 1.56 (3H, s), 1.55 (9H, s), 3.47−3.54 (2H, m), 4.04 (1H, m), 4.85−4.92 (2H, m, $J = 3.3$ Hz), 5.37 (1H, dd, $J = 6.3$ Hz), 5.90 (1H, d, $J = 2.3$ Hz), 7.05 (2H, bs), 8.13 (1H, s); ^{13}C NMR ($CDCl_3$) δ 25,49, 27.68, 27.99, 63.41, 81.19, 81.26, 85.16, 87.00, 89.11, 102.08, 11.94, 147.12, 147.87, 149.04, 149.76, 153.07. Anal. Calcd for $C_{18}H_{25}N_5O_7$: C, 51.06; H, 5.95; N, 16.54. Found: C, 50.74; H, 5.75; N, 16.14.

***N*^{7}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-(Methyl *N*,*N*-Diisopropylphosphoramidite) (27).** A mixture of *N*^{7}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (**26**) (423 mg, 1.0 mmol) and diisopropylammonium tetrazolide (111 mg, 0.65 mmol) were dried by repeated coevaporation three times with dry pyridine and

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twice with dry toluene and finally dissolved in dry CH_2Cl_2 (10 mL). To this solution was added methyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (373 μL, 1.3 mmol). After being stirred under argon atmosphere at room temperature 1 h, the mixture was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with hexane−ethyl acetate (9:1, v/v) containing 1% triethylamine. The fractions containing **27** were combined and concentrated to give **27** (410 mg, 70%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ 149.03, 149.29; ^{1}H NMR ($CDCl_3$) δ 1.09−1.16 (12H, m), 1.35 (3H, s), 1.56 (3H, s), 1.63 (9H, s), 3.36−3.42 (3H, 2d), 3.53−3.79 (4H, m), 4.33 (1H, m), 5.00−5.03 (1H, m), 5.49 (1H, m), 6.10 (1H, s), 8.18 (1H, 2s); ^{13}C NMR ($CDCl_3$) δ 22.98, 23.01, 2.51, 24.60, 24.62, 24.71, 25.31, 25.56, 25.61, 27.26, 28.00, 42.67, 42.84, 45.25, 50.62, 50.66, 50.88, 50.92, 62.95, 63.16, 63.42, 82.33, 82.38, 86.36, 86.44, 86.47, 86.58, 87.27, 87.31, 101.96, 101.99, 113.70, 113.76, 147.86, 147.88, 147.96, 148.68, 149.88, 153.54, 153.57. Anal. Calcd for $C_{24}H_{41}N_6O_3P$: C, 51.36; H, 7.07; N, 14.38. Found: C, 51.25; H, 7.32; N, 14.17.

***N*^{7}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Methyl *N*-(*N*-Trityl-L-prolyl)phosphoramidate] (28).** A mixture of *N*-Tr-L-prolinamide (**17**) (143 mg, 0.4 mmol) and phosphoramidite **27** (351 mg, 0.6 mmol) were dried by repeated coevaporation three times each with dry pyridine and with dry toluene and finally dissolved in dry CH_2Cl_2−dry MeCN (6 mL, (1:1, v/v)). The solution was added to 5-(3,5-dinitrophenyl)-1*H*-tetrazole (283 mg, 1.2 mmol), which was dried by repeated coevaporation with dry pyridine and dry toluene. After the mixture was stirred at room temperature for 10 min, *tert*-butyl hydroperoxide (249 μL, 2.0 mmol) was added. After being stirred at room temperature for 5 min, the mixture was diluted with $CHCl_3$ and washed three times with H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness under reduced pressure. The residue was treated with 2 M iodine (6 mL, pyridine−H_2O, (9:1, v/v)) at room temperature for 30 min. The reaction mixture was diluted with $CHCl_3$ and washed three times with sat. sodium thiosulfate. The organic layer was dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure. The residue was applied to a silica gel column, and elution was performed with $CHCl_3$−MeOH (99:1, v/v) containing 1% pyridine. The fractions containing **28** were combined and concentrated to give **28** (92 mg, 27%) as a colorless foam: ^{31}P NMR ($CDCl_3$) δ −0.48, −0.40; ^{1}H NMR ($CDCl_3$) δ 1.09−1.17 (12H, m), 1.35 (3H, s), 1.56 (3H, s), 1.63 (9H, s), 3.36−3.42 (3H, dd, J_{PH} = 12.9 Hz, J = 4.3 Hz,), 3.53−3.79 (4H, m), 4.33 (1H, m), 5.01 (1H, dd, J = 3.3 Hz, J = 6.3 Hz), 5.49 (1H, m), 6.19 (1H, s), 8.18 (1H, 2s); ^{13}C NMR ($CDCl_3$) δ 21.51, 24.30, 24.35, 25.52, 27.20, 28.00, 31.69, 31.75, 50.70, 50.79, 54.30, 54.39, 54.42, 54.46, 54.51, 65.57, 65.69, 67.30, 67.39, 78.26, 81.25, 81.80, 81.99, 82.79, 82.98, 85.15, 85.57, 85.68, 85.80, 86.65, 86.68, 87.01, 87.10, 102.03, 113.92, 113.98, 125.16, 126.55, 127.83, 127.85, 128.09, 128.89, 129.08, 129.10, 143.90, 147.55, 147.60, 148.05, 148.73, 148.77, 149.83, 153.58, 166.45, 177.35, 177.40.

Phosmidosine (1). Compound **28** (85.6 mg, 0.1 mmol) was treated with 80% formic acid (1 mL) at room temperature for 12 h. The mixture was diluted with H_2O and washed once with $CHCl_3$. The aqueous layer was concentrated and coevaporated three times with H_2O to remove formic acid. The residue was purified by C-18 reversed-phase silica gel column chromatography (H_2O:MeCN = 98:2−96:4) to give **1** (33 mg, 70%) as a white powder. The two isomers were additionally separated by preparative HPLC. **Isomer A (1a).** ^{31}P NMR (D_2O) δ 11.89; ESI-MS *m*/*z* Calcd for $C_{16}H_{25}N_7O_8P$, 474.1502; Observed (M + H) 474.1494. **Isomer B (1b).** ^{31}P NMR (D_2O) δ 12.42; ESI-MS *m*/*z* Calcd for $C_{16}H_{25}N_7O_8P$, 474.1502; Observed (M + H) 474.1501.

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