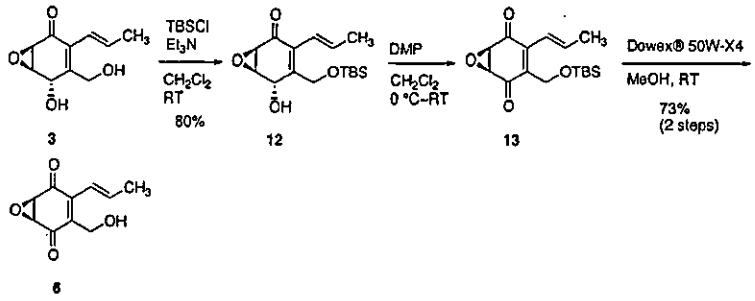
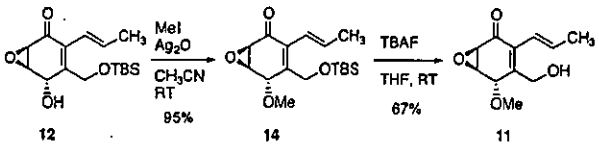
SCHEME 3. Preparation of Monomer 6



SCHEME 4. Preparation of Monomer 11



oxidation with MnO_2[17] gave 3-(*tert*-butyldimethylsiloxymethyl)-2-cyclohexen-1-one (**21**). The desired 3-hydroxymethyl-2-propenyl-2-cyclohexen-1-one (**10**) was obtained by α-iodination of ketone **21**, a Suzuki coupling reaction, and deprotection via the method developed for the synthesis of monomer **3**.[3,5]

Experimental Results for the Oxidation/6π-Electrocyclization/Diels–Alder Reaction. The results for the oxidation/6π-electrocyclization/Diels–Alder reaction of the monomers **6**, **10**, **3**, and **11** are summarized in Schemes 6–9, respectively. Though oxidation of alcohol **6** with MnO_2 did not proceed owing to the neighboring electron-withdrawing substituent, **6** was smoothly oxidized within 15 min by DMP in $CDCl_3$. ^1H NMR (400 MHz) showed that 2*H*-pyran **8** was formed while the corresponding aldehyde **7** could not be detected, and that epoxyquinol A-type product **9** had been formed in 50% yield with some 2*H*-pyran **8** remaining. When the crude reaction mixture was left neat for 1 h, 2*H*-pyran **8** was completely converted to the epoxyquinol A-type product **9** in 70% yield without formation of any other diastereomers (Scheme 6). These results indicate that the 6π-electrocyclization is fast, and that aldehyde **7** is readily converted to 2*H*-pyran **8**. The Diels–Alder reaction is also a fast process, proceeding only via the *endo-anti*(epoxide)-*anti*(Me)-hetero mode to afford epoxyquinol A-type adduct **9** in good yield. Although 2*H*-pyran **8** could be regarded as a poor diene because the two electron-withdrawing groups would decrease its HOMO energy, the dimerization is fast, which indicates that **8** acts as a reactive dienophile in the Diels–Alder reaction.

The reaction profile of cyclohexenone monomer **10** is rather different from that of epoxyquinone **6**. Unlike epoxyquinone **6**, the oxidation of **10** proceeds efficiently with MnO_2, and the ^1H NMR spectrum of the reaction suggests the presence of aldehyde **23**, 2*H*-pyran **24** not being observed. Generation of the Diels–Alder adduct **25** was slow, and aldehyde **23** was gradually converted into epoxyquinol A-type product **25** without detection of the 2*H*-pyran intermediate **24**. Eventually epoxyquinol A-type product **25** was gradually formed in 70% yield without formation of epoxyquinol B-type product after aldehyde **23** was allowed to stand neat for 10 h (Scheme 7). These results indicate that formation of the dimerized product is slow, and that only the *endo-anti*(Me)-hetero mode occurs. This phenomenon, namely, that the observed intermediate (aldehyde or 2*H*-pyran) is completely different for the reactions of epoxyquinone **6** and cyclohexenone **10**, is quite puzzling (vide infra).

The oxidation of epoxyquinol **3** was successfully carried out using MnO_2 without protection of the secondary alcohol.[3] The intermediate aldehyde **4** thus generated was not detected but was rapidly and smoothly converted to 2*H*-pyran **5**. The Diels–Alder dimerization takes 4 h to go to completion, and epoxyquinols A and B are formed in yields of 40% and 25%, respectively (Scheme 8). 6π-Electrocyclization is a fast process, and the Diels–Alder reaction is slower than that of the epoxyquinone **6**. The reaction proceeds via both *endo-anti*(epoxide)-*anti*(Me)-hetero and *exo-anti*(epoxide)-*anti*(Me)-homo modes, generating both epoxyquinols A and B, while no other diastereomers are formed.

The methoxy derivative **11**, however, gave results quite different from those of epoxyquinol **3**. When **11** was oxidized with MnO_2, aldehyde **26**, which was not detected by ^1H NMR, was smoothly and completely converted into 2*H*-pyran derivatives **27** in a 4.5:1 diastereomer ratio after 1.5 h, although which isomer predominates has not been determined. As the Diels–Alder reaction of methoxy-2*H*-pyran **27** does not proceed even under more forcing reaction conditions, the single process of 6π-electrocyclization can be monitored in this system. The diastereomer ratio of **27** changed from 4.5:1 to 1.2:1 after 10 h (Scheme 9). This result clearly indicates the existence of an equilibrium[18] between *anti*- and *syn*-2*H*-pyrans **27**.

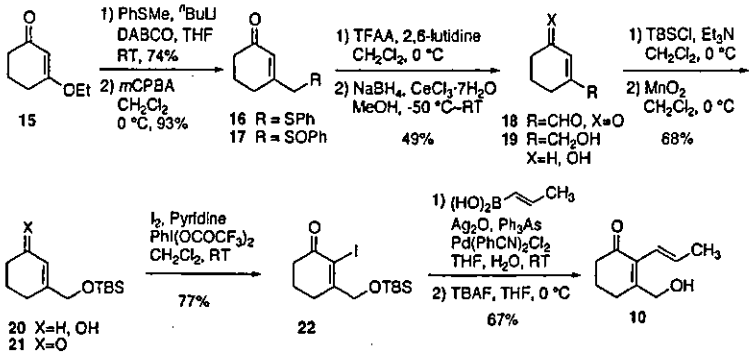
The present oxidative dimerization is composed of the three successive cascade reactions oxidation, 6π-electrocyclization, and Diels–Alder dimerization. Oxidation of the primary alcohol proceeds smoothly for all the substrates examined, while the next two reactions are dependent on the substituents. The 6π-electrocycylization and Diels–Alder reactions were separately investigated using theoretical calculations.

Theoretical Study of the 6π-Electrocyclization. Theoretical calculations were carried out to understand the reaction profile of the 6π-electrocyclization and Diels–Alder dimerization. The geometries of all stationary points were fully optimized at the B3LYP/6-31G*

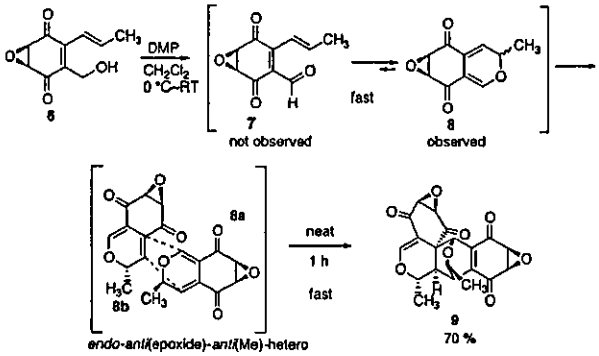
(17) Aoyama, T.; Sonoda, N.; Yamauchi, M.; Toriyama, K.; Anzai, M.; Ando, A.; Shioiri, T. *Synlett* **1998**, 35.

(18) Marvell. E. N. *Thermal Electrocyclic Reactions*; Academic Press: New York, 1980.

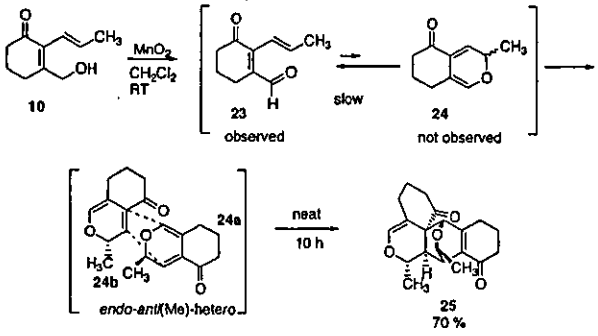
SCHEME 5. Preparation of Monomer 10



SCHEME 6. Dimerization of Epoxyquinone 6



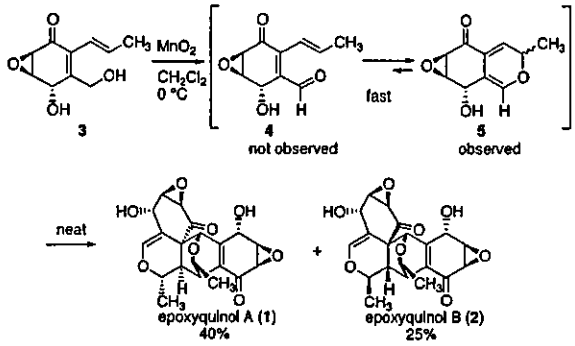
SCHEME 7. Dimerization of Cyclohexenol 10



level, and the properties of the molecules were also calculated at the same level.[19] All points were characterized as minima or saddle points by calculation of the harmonic vibrational frequencies, using analytical second derivatives.

Rodriguez-Otero has studied a series of 6π-electrocyclizations of (*Z*)-hexa-1,3,5-triene and its heterosubstituted analogues at various levels of theory and found that the reaction is slightly endothermic, the required TS energy for the 6π-electrocyclization of (2*Z*)-2,4-pentadienal being calculated as 21.52 kcal/mol at the B3LYP/6-31G**//B3LYP/6-31G** level.[20] Porco et al. studied the 6π-electrocyclization of epoxyquinone derivatives in their torreyanic acid synthesis. Their computational study indicates that this reaction is highly exothermic and that the TS energy is 5.0 kcal/mol for the *syn*-isomer and 10.2 kcal/mol for the *anti*-isomer at the B3LYP/6-31G*//AM1 level.[9b] As there has been no systematic study of substituent effects on 6π-electrocyclizations,[18] the 6π-electrocyclizations of the methyl ether epoxyquinol derivative **26**, epoxyquinone **7**, epoxyquinol **4**, and cyclohexenone **23** have been investigated in detail. Scheme 10 represents the transition structures leading to both *syn*- and *anti*-2*H*-pyrans along with the TS energies and distances of the newly formed O_1-C_2 bond (2*H*-pyran numbering).

SCHEME 8. Dimerization of Epoxyquinol Monomer 3



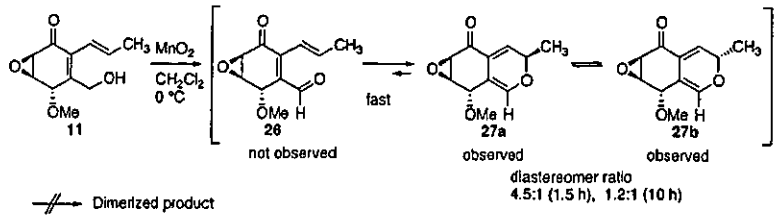
The methoxy derivative **26** is an ideal substrate as there is no subsequent Diels–Alder reaction and hence the electrocyclization itself can easily be monitored experimentally. Therefore, the 6π-electrocyclization of this compound was examined first. 6π-Electrocyclization of **26** is exothermic, and both *syn*- and *anti*-2*H*-pyrans **27** are calculated to be more stable than the parent aldehyde **26**, by 4.18 and 4.16 kcal/mol, respectively. The TS energies leading to the two diastereomers of the 2*H*-pyran are low (15.50 and 17.74 kcal/mol), and the TS energies of retro-6π-electrocyclization are under 22 kcal/mol (19.68 and 21.90 kcal/mol). The TS energy for the reaction leading to *syn*-2*H*-pyran **27a** is lower than that of the reaction leading to *anti*-isomer **27b**, while the *anti*- and *syn*-isomers have the same stability.

These calculations are in good agreement with the experimental results as follows: (1) After oxidation, 2*H*-pyran **27** was observed without detection of intermediate aldehyde **26**. This is because on formation aldehyde **26** was immediately converted into 2*H*-pyran **27** as the TS energy is low and 6π-electrocyclization is exothermic. (2)

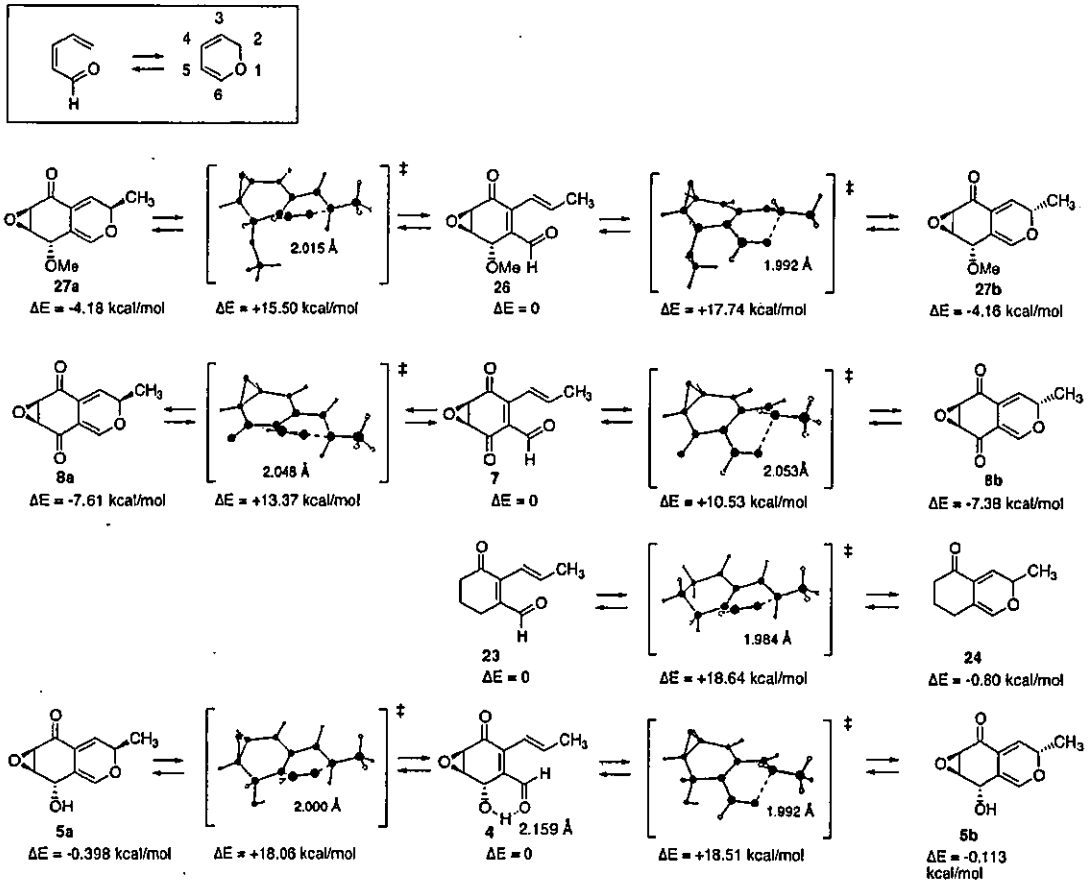
(19) All calculations were performed with the program package TITAN 1.0.5 of Schrodinger, Inc. (http://www.schrodinger.com) and Wavefunction Inc. (http://www.wavefun.com).

(20) Rodriguez-Otero, J. *J. Org. Chem.* **1999**, *64*, 6842.

SCHEME 9. 6π-Electrocyclization of 11



SCHEME 10. Theoretical Calculations on the 6π-Electrocyclization



Though the major isomer of **27** has not been determined, the diastereomer ratio of 2*H*-pyrans **27a,b** changed from 4.5:1 (1.5 h) to 1.2:1 (10 h). We can surmise that this is because of an equilibrium occurring between 2*H*-pyrans **27a,b** and aldehyde **26**, a result of the low TS energies of both the 6π-electrocyclization and its retro reaction.

As calculated results for the methoxy derivative **26** proved to be in good agreement with experimental findings, the other 6π-electrocyclizations of **4**, **7**, and **23** have also been examined, and the following noteworthy features have been found from comparison of the four reactions: (1) The lone pair of the formyl oxygen is involved to a great extent in the TS as shown by the loss of planarity in the dihedral angles of $C_2O_1C_6C_5$ (2*H*-pyran numbering), and this is consistent with the calculations of Rodriguez-Otero on the parent 2,4-pentadienal.[20] (2) As the TS energies of both the 6π-electrocyclization and retro-6π-electrocyclization are below 22 kcal/mol for all the substrates, there is equilibrium among the *anti*- and *syn*-2*H*-pyrans and aldehyde at rt. (3) As the substituent becomes more electron-withdrawing, the TS energy becomes lower, indicating that 6π-electrocyclization becomes easier. (4) The order of the length of the newly formed O_1-C_2 bond in the transition state is epoxyquinone **7** > epoxyquinol **4** = methoxy derivative **26** > cyclohexenone **23**. As the substituent becomes more electron-withdrawing, the length O_1-C_2 in the TS becomes longer, indicating that the new bond has formed to a considerably lesser extent, and that the transition state is closer to the starting material. (5) As the substituent becomes more electron-withdrawing, the reaction becomes more exothermic, and the stability of the 2*H*-pyran over the aldehyde increases except in the case of epoxyquinol **4**. (6) The reaction of epoxyquinol **4** is a special case, in which aldehyde **4** and 2*H*-pyrans **5a,b** are of almost the same energy, while for the corresponding methoxy derivative 2*H*-pyrans **27a,b** are more stable than aldehyde **26** by ca. 4 kcal/mol. This is because there

TABLE 1. TS Energy of the Reaction Modes of Dimerization of Epoxyquinone 8

entry	reaction mode	diene	dienophile	TS energy/kcalmol^{-1}
1	*endo-anti*(epoxide)-*anti*(Me)-hetero	**8a**	**8b**	13.52
2	*endo-syn*(epoxide)-*syn*(Me)-hetero	**8a**	**8b**	27.55
3	*exo-syn*(epoxide)-*syn*(Me)-hetero	**8a**	**8b**	18.76
4	*exo*-anti(epoxide)-anti(Me)-hetero	**8a**	**8b**	17.24
5	*exo-anti*(epoxide)-*anti*(Me)-homo	**8a**	**8a**	15.43
6	*exo-syn*(epoxide)-*syn*(Me)-homo	**8a**	**8a**	20.56
7	*endo-anti*(epoxide)-*anti*(Me)-homo	**8a**	**8a**	25.04
8	*endo-syn*(epoxide)-*syn*(Me)-homo	**8a**	**8a**	18.38
9	*exo-anti*(epoxide)-*syn*(Me)-homo	**8b**	**8b**	19.04
10	*exo-syn*(epoxide)-*anti*(Me)-homo	**8b**	**8b**	15.93
11	*endo-syn*(epoxide)-*anti*(Me)-homo	**8b**	**8b**	20.40
12	*endo-anti*(epoxide)-*syn*(Me)-homo	**8b**	**8b**	21.79
13	*endo-anti*(epoxide)-*syn*(Me)-hetero	**8b**	**8a**	23.19
14	*endo-syn*(epoxide)-*anti*(Me)-hetero	**8b**	**8a**	18.60
15	*exo-syn*(epoxide)-*anti*(Me)-hetero	**8b**	**8a**	18.25
16	*exo-anti*(epoxide)-*syn*(Me)-hetero	**8b**	**8a**	20.98

TABLE 2. Frontier Orbital Energies of 8, 24, and 5

entry	orbital	orbital energy/eV	entry	orbital	orbital energy/eV
1	HOMO of **8a**	−6.22730	6	LUMO of **24**	−1.60257
2	LUMO of **8a**	−2.25359	7	HOMO of **5a**	−5.80672
3	HOMO of **8b**	−6.25759	8	LUMO of **5a**	−1.97206
4	LUMO of **8b**	−2.27970	9	HOMO of **5b**	−5.88086
5	HOMO of **24**	−5.46902	10	LUMO of **5b**	−1.99471

SCHEME 11. Reaction Modes of 2*H*-Pyran

is a hydrogen-bond interaction between the hydroxy group and formyl group in **4** (2.159 Å) as shown in Scheme 10, and this stabilizes the aldehyde **4**, so a higher TS energy is required for the conversion into 2*H*-pyrans **5** than for conversion of the corresponding methyl ether **26** into **27**.

Theoretical Study of the Diels–Alder Dimerization. Theoretical calculations on the homo-Diels–Alder reaction of 2*H*-pyran indicate the regiochemistry should be one of those shown in Scheme 11 according to the frontier orbital theory.[21,22] For this regiochemistry, there are 16 possible reaction modes[23] of the Diels–Alder reaction of both epoxyquinone **6** and epoxyquinol **3**. Of these, only the *endo-anti*(epoxide)-*anti*(Me)-hetero mode is observed with epoxyquinone **6**, while both the *endo-anti*(epoxide)-*anti*(Me)-hetero and *exo-anti*(epoxide)-*anti*(Me)-homo modes are detected with epoxyquinol **3**. In the case of cyclohexenone **10**, of the eight possible reaction modes[24] only the *endo-anti*(Me)-hetero mode was observed.

All 16 reaction modes for epoxyquinone **6** (**8a,b**) were investigated by theoretical calculations, and the TS energies of these reaction modes are summarized in Table 1. The frontier orbital energies of 2*H*-pyran derivatives **8a,b**, **24**, and **5a,b** and the HOMO–LUMO energy gap of **8a,b** have been summarized in Tables 2 and 3, respectively.

TABLE 3. Energy Gap of Frontier Orbitals of 8

entry	frontier orbital	energy gap/ eV
1	HOMO of **8a**–LUMO of **8a**	3.9737
2	HOMO of **8a**–LUMO of **8b**	3.9476
3	HOMO of **8b**–LUMO of **8b**	3.9779
4	HOMO of **8b**–LUMO of **8a**	4.0040

Calculations indicate that the orientation of the methyl groups is very important. That is, the three reaction modes with the lowest TS energy are the *endo-anti*-(epoxide)-*anti*(Me)-hetero (Table 1, entry 1), *exo-anti*-(epoxide)-*anti*(Me)-homo (entry 5), and *exo-syn*(epoxide)-*anti*(Me)-homo (entry 10) modes, in which the two methyl groups are oriented on opposite sides of the approaching dienophile and diene. That is to say, the steric hindrance caused by the methyl groups is so large that the methyl group of the diene monomer should be oriented *anti* to its reacting face, and that of the dienophile monomer oriented *anti* to its reacting face; otherwise the TS energies are over 16 kcal/mol.

Of the 16 reaction modes, the TS energy of the *endo-anti*(epoxide)-*anti*(Me)-hetero mode is found to be the lowest, 13.52 kcal/mol, indicating that this reaction is a facile process (entry 1). Scheme 12 indicates that steric hindrance is minimized through the two methyl groups, occupying axial positions. This is orbitally preferable in terms of the lowest HOMO–LUMO energy gap (Table 3, entry 2), and also because of its secondary orbital interactions (*endo* rule). The next lowest reaction mode is *exo-anti*(epoxide)-*anti*(Me)-homo, which would lead to the epoxyquinol B-type product (Table 1, entry 5). As this second lowest TS energy is 15.43 kcal/mol, which is 1.91

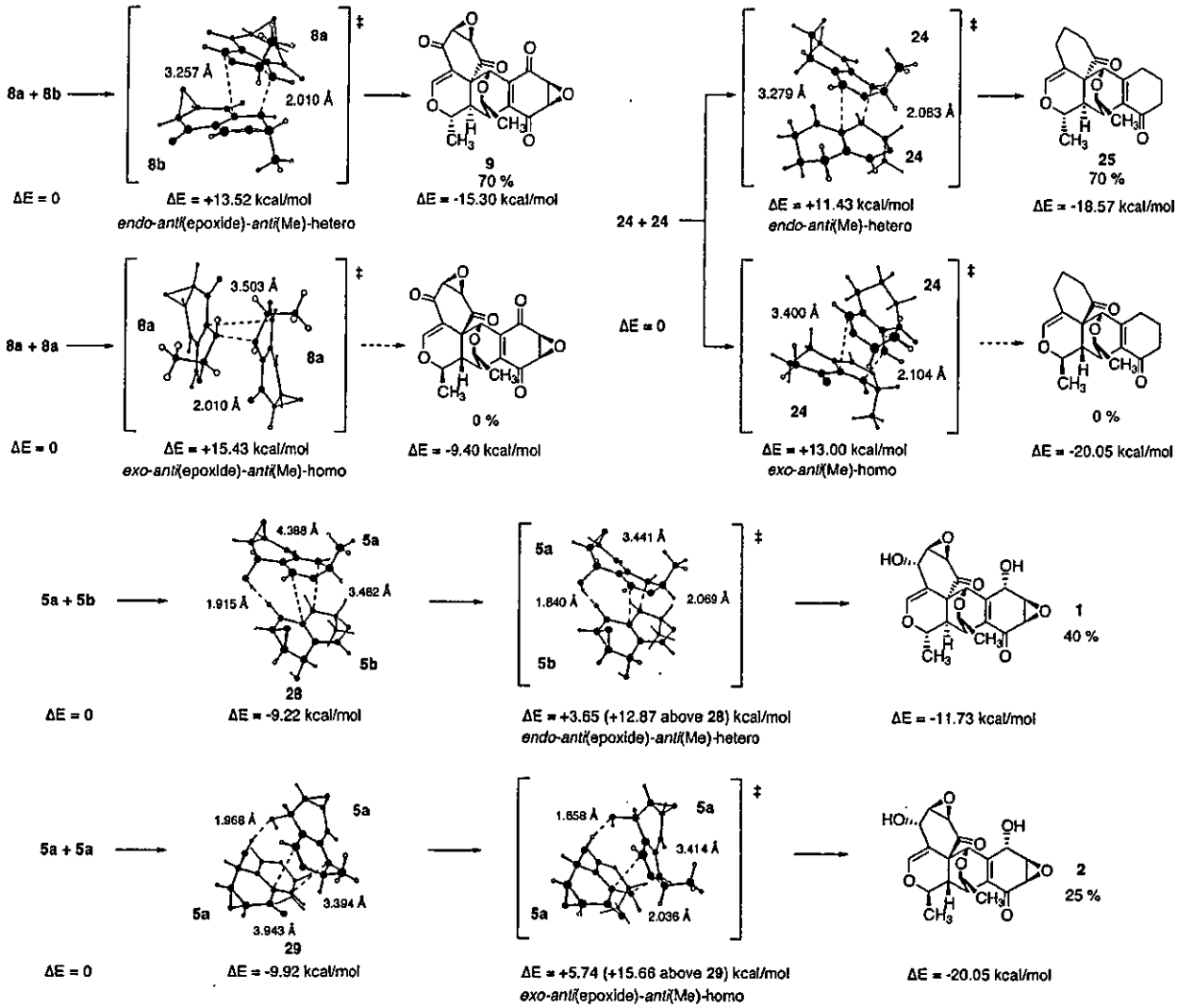
(21) Fleming, I. *Frontier Orbitals and Organic Chemical Reactions*; John Wiley & Sons: Chichester, U.K., 1976.

(22) The coefficients of the HOMO and LUMO energies of the 2*H*-pyrans are described in the Supporting Information.

(23) The 16 possible reaction modes are as follows: *endo-anti*-(epoxide)-*anti*(Me)-hetero, *exo-anti*(epoxide)-*anti*(Me)-homo, *endo-anti*(epoxide)-*anti*(Me)-homo, *exo-anti*(epoxide)-*anti*(Me)-hetero, *endo-anti*(epoxide)-*syn*(Me)-hetero, *exo-anti*(epoxide)-*syn*(Me)-homo, *endo-anti*-(epoxide)-*syn*(Me)-homo, *exo-anti*(epoxide)-*syn*(Me)-hetero, *endo-syn*-(epoxide)-*anti*(Me)-hetero, *exo-syn*(epoxide)-*anti*(Me)-homo, *endo-syn*-(epoxide)-*anti*(Me)-homo, *exo-syn*(epoxide)-*anti*(Me)-hetero, *endo-syn*-(epoxide)-*syn*(Me)-hetero, *exo-syn*(epoxide)-*syn*(Me)-homo, *endo-syn*-(epoxide)-*syn*(Me)-homo, *exo-syn*(epoxide)-*syn*(Me)-hetero.

(24) Because there is no epoxide in **10**, the number of possible reaction modes is reduced from sixteen in **3** to eight.

SCHEME 12. Theoretical Calculation of the Diels–Alder Reaction



kcal/mol higher than that of the lowest, the reaction should not proceed via this mode but the via *endo-anti*(epoxide)-*anti*(Me)-hetero mode alone, which is consistent with the experimental result that epoxyquinol A-type dimer **9** was selectively obtained.

In the case of cyclohexenone **10** (**24**), two reaction modes, which would lead to epoxyquinol A- and B-type products, were investigated (Scheme 12). The TS energy of the *endo-anti*(Me)-hetero mode (11.43 kcal/mol) is lower than that of the *exo-anti*(Me)-homo mode (13.00 kcal/mol), which is consistent with the experimental result that the *endo-anti*(Me)-hetero dimer **25** (epoxyquinol A-type product) was selectively formed.

The TS energies of the 6π-electrocyclization and the Diels–Alder reaction of **3**, **6**, and **10** are summarized in Table 4. By comparing epoxyquinone **6** and cyclohexenone **10**, the aforementioned puzzling experimental results can be reasonably explained as follows: As described previously, only 2*H*-pyran **8**, but not aldehyde **7**, was detected for epoxyquinone **6**, while only aldehyde **23**, but not 2*H*-pyran **24**, was observed for cyclohexenone **10**. These contrasting results are due to the different reaction

TABLE 4. TS Energies of the 6π-Electrocyclization and Diels–Alder Reaction

	TS energy/kcalmol^{-1}	
substrate	6π-electrocyclization	Diels–Alder reaction
epoxyquinone **6** (**8**)	10.53,[a] 13.37[b]	13.52
cyclohexenone **10** (**24**)	18.64	11.43
epoxyquinol **3** (**5**)	18.51,[a] 18.06[b]	12.87,[c] 15.66[d]

[a] The TS energy to *anti*-2*H*-pyran. [b] The TS energy to *syn*-2*H*-pyran. [c] The TS energy to **1**. [d] The TS energy to **2**.

profiles of these two reactions. That is, the rate-determining step has been reversed: The rate-determining step for epoxyquinone **6** is the Diels–Alder reaction, while that for cyclohexenone **10** is 6π-electrocyclization (Table 4). For the 6π-electrocyclization, the TS energy for epoxyquinone **6** is lower than that for cyclohexenone **10** because the TS energy becomes lower as the substituent becomes more electron-withdrawing, and there are two electron-withdrawing groups in **6** compared with only one in **10**. On the other hand, the TS energy of the Diels–Alder reaction of epoxyquinone **6** is higher than that of

cyclohexenone **10** because there is steric hindrance caused by the epoxide in **6**, and also because the Diels–Alder reaction of **10** is orbitally favorable. Namely, the two electron-withdrawing groups reduce the reactivity of the diene **8** by lowering its HOMO energy, and cause a larger HOMO–LUMO energy gap for **8a/8b** (3.9476 eV, Table 3, entry 2) than for **24** (3.8665 eV, Table 2, entry 5 (HOMO of **24**), entry 6 (LUMO of **24**)). As a result, the rate-determining step has been reversed.

Compared with the above two substrates, the epoxyquinol **3** (**5a,b**) had a different profile: Though the two 2*H*-pyran monomers **8** and **24** react to afford dimerized product, monomer **5** from epoxyquinol **3** was not directly transformed into the Diels–Alder products **1** and **2**. Calculations suggest that initially the two monomers **5a,b** preassociate to give intermediate complexes **28** and **29**, which are more stable than the parent **5a** + **5b** and **5a** + **5a** by 9.22 and 9.92 kcal/mol, respectively. This stabilization can be ascribed to a hydrogen-bond interaction as shown in Scheme 12. The hydroxy group of **5a** coordinates the carbonyl lone pair of **5b** in **28**, at a distance of 1.915 Å, while two OH groups of two different **5a** molecules interact with each other in **29**, at a distance of 1.968 Å. From the intermediates **28** and **29**, the dimerization proceeds to afford Diels–Alder products **1** and **2**. As the TS energies for the *endo-anti*(epoxide)-*anti*(Me)-hetero and *exo-anti*(epoxide)-*anti*(Me)-homo modes are 12.87 and 15.66 kcal/mol, respectively, the former mode would be theoretically more favorable than the latter, and this is consistent with the experimental result that **1** was formed predominantly. However, the large difference between the TS energies of the two modes (2.79 kcal/mol) is not in good agreement with the experiment, in which **2** was also formed in 25% yield. This discrepancy could be the result of neglecting solvent effects in the calculation, which would be detrimental owing to the existence of hydrogen-bonding. The hydrogen-bonding effect is found to be operative not only in the ground state, but also in the transition state. As shown in Scheme 12, the hydrogen-bond activates the ketone function in the *endo-anti*(epoxide)-*anti*(Me)-hetero mode, whereas there is hydrogen-bonding stabilization of the TS in the *exo-anti*(epoxide)-*anti*(Me)-homo mode.

This hydrogen-bonding interaction, which is found to be important in the transition state in the theoretical calculations, is also found in the crystal structure of the final product, epoxyquinol B, in which a hydrogen-bond between the two OH groups has in fact been observed.[25] Moreover, if these transition-state hydrogen-bonds exist, the distribution of epoxyquinols A and B should be affected by the solvent, which is found to be the case. As shown in Table 5, **1** was formed predominantly in neat conditions or in benzene solution, while **2** was the major product in toluene and CH_2Cl_2. Lewis acids such as $LiClO_4$[26] accelerate the reaction, affording epoxyquinol A predominantly via the orbitally preferable *endo* mode and in short reaction time. This is in a marked contrast with the dimerization of cyclohexenone **10**, in which the product distribution is not affected at all by the solvent (Table 6). That is, epoxyquinol A-type product **25** was selectively obtained as the sole product, irrespective of the solvent. Another interesting observation is that the reaction of **10** in MeOH is much faster than that in benzene, toluene, and CH_2Cl_2, while the reaction of epoxyquinol **3** is slower in MeOH than that in benzene and toluene. Hydrogen-bonding activation by MeOH, which has been observed in the reaction of **10**, cannot be realized in the reaction of **3** owing to strong intermolecular hydrogen-bonding. This is further evidence for the importance of hydrogen-bonding in the oxidative dimerization of **3**. Moreover, the predominant formation of epoxyquinol B in toluene is synthetically useful, because epoxyquinol B is a more potent angiogenesis inhibitor than epoxyquinol A.[2]

TABLE 5. Solvent Effect on the Oxidative Dimerization of 3

entry	solvent	time/h	yield[a]/% 1	yield[a]/% 2
1	neat	4	40	25
2	$LiClO_4/Et_2O$	2.5	46	25
3	benzene	12	39	32
4	toluene	12	25	45
5	CH_2Cl_2	33	21	38
6	Et_2O	46	25	21
7	MeOH	94	21	21
8	CH_3CN	140	14	21

[a] Isolated yield.

TABLE 6. Solvent Effect on the Oxidative Dimerization of 10

entry	solvent	time/h	yield[a]/%
1	neat	10	70
2	MeOH	25	73
3	benzene	43	30
4	toluene	43	35
5	CH_2Cl_2	72	25

[a] Isolated yield of **25**.

The importance of the hydroxy group is also demonstrated by the following experiment. Diels–Alder dimerization of the methyl ether **11**, in which there is no hydrogen-bonding interaction, does not proceed, though the 6π-electrocyclization does (vide supra, Scheme 9). This is another piece of evidence supporting the importance of the hydroxy group in the dimerization of epoxyquinol **3**. The steric hindrance caused by the methoxy groups of **27** would prevent the Diels–Alder reaction.

There are literature precedents in which intermolecular hydrogen-bonding can be successfully utilized for the control of the stereochemistry of a Diels–Alder reaction.[27] In this oxidative dimerization, nature has also successfully employed hydrogen-bonding in the Diels–Alder reaction for the formation of epoxyquinol B.

Conclusions

In the oxidative dimerization of epoxyquinone **6** and cyclohexenone **10**, the preferred reaction modes are *endo-*

(25) See footnote 18 of ref 6.
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anti(epoxide)-*anti*(Me)-hetero and *endo-anti*(Me)-hetero, respectively, while both epoxyquinols A and B are formed via the *endo-anti*(epoxide)-*anti*(Me)-hetero and *exo-anti*-(epoxide)-*anti*(Me)-homo modes in the dimerization of epoxyquinol **3** because of intermolecular hydrogen-bonding, which has been proved to exist by theoretical calculations and several experimental results. Other noteworthy features are as follows: The existence of an equilibrium between the 2*H*-pyran and aldehyde has been theoretically and experimentally demonstrated in the case of the methoxy derivative **11**. In the dimerization of epoxyquinol **3**, monomer 2*H*-pyrans **5** preassociate to afford complexes **28** and **29**, from which the Diels–Alder reaction proceeds. Theoretical calculations have also clarified the difference in reaction profiles between epoxyquinone **6** and cyclohexenone **10**. Namely, the rate-determining step of the former is the Diels–Alder reaction, while that of the latter is 6π-electrocyclization.

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Supporting Information Available: Complete experimental procedures, full characterization, copies of ^{1}H and ^{13}C NMR and IR of all new compounds, and Cartesian coordinates for calculated transition states of 6π-electrocyclization and Diels–Alder reaction (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JOC*Article*

Synthesis of Chemically Stabilized Phosmidosine Analogues and the Structure–Activity Relationship of Phosmidosine

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Phosmidosine is known to have potent antitumor activity and the unique property of stopping cell growth at the G_1 phase in the cell cycle. However, this natural product having *N*-prolylphosphoramidate and *O*-methyl ester linkages on the 5′-phosphoryl residue is unstable under basic conditions and even during the chemical synthesis due to its inherent methyl transfer activity. To find stable derivatives of phosmidosine, a variety of phosmidosine analogues **1a–d** replaced by longer alkyl groups in place of the methyl group on the phosphoramidate linkage were synthesized by reaction of alkyl *N*-(*N*-tritylprolyl)phosphorodiamidite derivatives **7a–d** with an 8-oxoadenosine derivative **4** protected with acid-labile protecting groups. Consequently, the *O*-ethyl ester derivative **1b** was found to be sufficiently stable in aqueous solution. When the prolyl group was replaced by other aminoacyl moieties, the reaction of *N*-tritylaminoacylamide derivatives **25a–d** with an appropriately protected 8-oxoadenosine 5′-(ethyl phosphoramidite) derivative **9** gave better results than the above coupling reaction. A phosphoramidothioate derivative **17** and several simple compounds such as **11**, **13**, and **15** lacking partial structures of phosmidosine were also synthesized. The antitumor activities of these modified analogues were extensively studied to clarify the structure–activity relationship of phosmidosine. As a result, the two diastereoisomers of longer alkyl-containing phosmidosine analogues both proved to have similar antitumor activities. Replacement of L-proline with other L-amino acids or D-proline resulted in considerable decrease of the antitumor activity. The non-nucleotidic materials **13** did not show any antitumor activity, but a simple core compound of **11** exhibited weak cytotoxicity. The phosphoramidothioate derivative **17** maintained essentially a similar antitumor activity, but the efficiency decreased slightly.

Introduction

Phosmidosine (**1a**) is an antibiotic having a unique *N*-acylphosphoramidate linkage. This natural product was first isolated by Uramoto et al. in 1991.[1] Later, its structure was finally determined by use of mass spectrometry.[2] Osada and co-workers reported that phosmidosine has biological activity capable of morphological reversion of temperature-sensitive v-src^{ts}NRK cells and stops the cell growth at the G_1 phase in the cell cycle.[3] The same research group also suggested that phosmidosine inhibits hyperphosphorylation of RB proteins by the action of RB-kinases as a result of the inhibition of cyclin D1 expression.[4] These intriguing properties led us to study the synthesis of phosmidosine and related compounds as potential candidates of new antitumor drugs.

We first reported the synthesis of a demethylated species (Phosmidosine B) of phosmidosine[5] and disclosed that it has significant antitumor activities in various cancer-related cell lines. Later, we also established an effective synthetic route to phosmidosine via an 8-oxoadenosine 5′-phosphoramidite derivative.[6] However, we encountered difficulty in synthesizing this final product in satisfactory yield. This is mainly because phosmidosine of the diester-type tends to decompose during its synthetic process, so the isolated yield decreases.

In this paper, we report the synthesis of chemically stabilized phosmidosine derivatives and the structure–

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[⊥] Kanazawa University.

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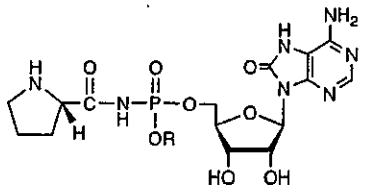

1a: R = Me (Phosmidosine)
1b: R =Et, **1c**: R = iPr, **1d**: R =Bu

FIGURE 1. Structure of phosmidosine and its stable analogues.

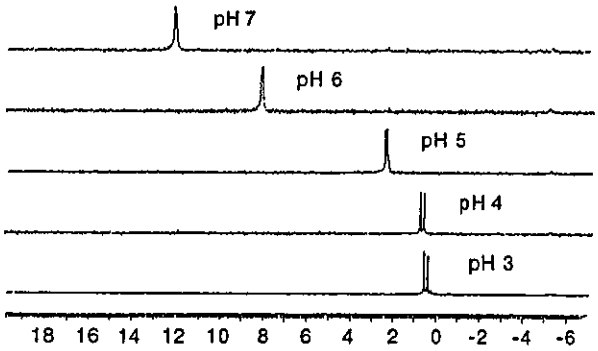

FIGURE 2. ^{31}P NMR spectra of a diastereomeric mixture of synthetic phosmidosine in citric–citrate buffer at pH 3–7.

activity relationship of phosmidosine based on comparison with the antitumor activities of phosmidosine-related compounds that lack structural elements or have other amino acids in place of the proline moiety.

Results and Discussion

Inherent Problems in the Synthesis of Phosmidosine. We encountered difficulty in obtaining phosmidosine without decomposition. Therefore, to understand what happened during the isolation process, we carefully examined the behavior of this compound in a citric acid–sodium citrate buffer with a pH range of 3–7 by use of ^{31}P NMR. As a result, it was found that the ^{31}P NMR resonance signals of a mixture of synthetic diastereomeric phosmidosines change dramatically upon change of the pH value of its solution. At pH 7, the diastereoisomers exhibited their ^{31}P NMR resonance signals at around 12 ppm but shifted to low-magnetic field at around 0 ppm, as shown in Figure 2.

The ^{31}P NMR signal change observed can be explained as follows. At pH 3, phosmidosine is protonated on the proline residue, as shown in form I of Figure 3, while at pH 7, phosmidosine exists as a zwitterion form II, as shown in Figure 3.

It was reported by McCloskey that under more basic conditions than pH 7, phosmidosine underwent rapid N–N phosphoryl rearrangement.[2] It was also reported that, heating of phosmidosine at pH 10 at 100 °C for 5 min resulted in a loss of 90% of its original activity, but when heating was conducted at pH 2 at 100 °C for 5 min, the decrease of the activity was suppressed to a degree of 20%.[1] From these results, phosmidosine is more stable in acidic media than in basic media. The demethylated derivative, phosmidosine B, as well as aminoacylamido-

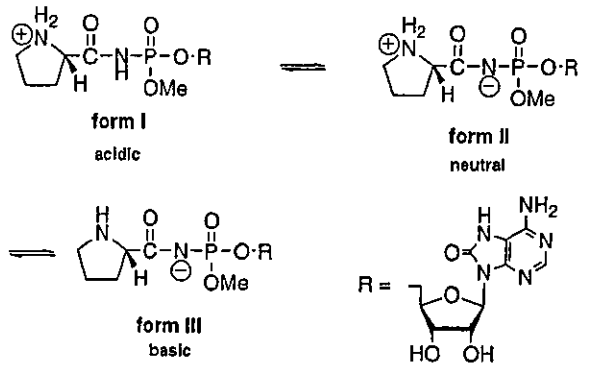

FIGURE 3. Possible structures of phosmidosine under acidic, neutral, and basic conditions.

AMP analogues,[7–9] are known to be quite stable under acidic and basic conditions. These compounds have commonly dissociated phosphate anions. It is likely that there are no more electrophilic centers because of the electron-donating effect of the phosphate oxy anion, leading to resistance to acids and bases. Therefore, the neutral original structure of phosmidosine is susceptible to nucleophiles such as water or its internal and external amino group, decomposing even under neutral conditions.

In particular, we observed that, when phosmidosine was diluted at pH 7 to a concentration prescribed for the ^{31}P NMR measurement, it remained intact for several days. However, once this material was condensed, considerable decomposition was observed. This is due not to the intramolecular N–N rearrangement of the phosphoryl group but rather to an intermolecular methyl transfer reaction.

In a concentrated solution, phosmidosine seems to transfer the methyl group intermolecularly to another phosmidosine molecule to give a mixture of the demethylated and methylated phosmidosine derivatives, as shown in path **a** of Figure 4. It is likely that the instability of phosmidosine is also due to susceptibility to not only intramolecular rearrangement (path **b**) resulting from the attack of the once-generated secondary amino group of the proline residue on the phosphorus atom but also intramolecular methyl transfer reaction (path **c**). All decomposition products described in Figure 4 were also observed and well characterized by McCloskey's extensive LC/MA studies on phosmidosine and its derivatives.[2] The sufficient stability of phosmidosine in its acidic solution can be explained since the prolyl amino group is completely protonated so that it loses the nucleophilic feature. Therefore, it is suitable to use acid-labile protecting groups during the synthesis of phosmidosine, and this material should be isolated as an ammonium salt.

Strategy for the Synthesis of Phosmidosine Analogues. On the basis of the above-mentioned discussion, we chose acid-labile protecting groups for the synthesis of proline and 8-oxoadenosine intermediates. The trityl group was chosen for the former, and the Boc and

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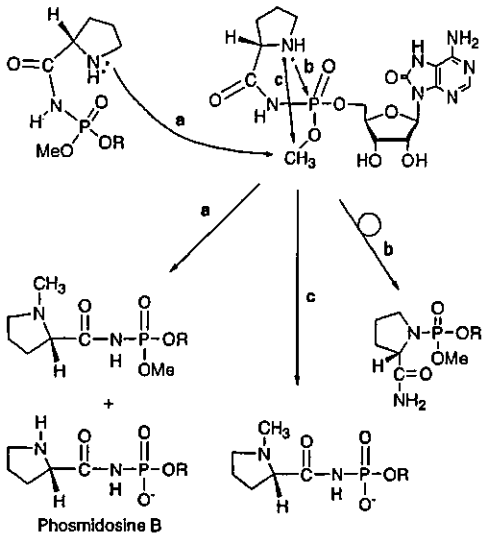

FIGURE 4. Intermolecular methyl transfer reaction (a) and intramolecular N–N rearrangement (b) of the phosphoryl group of phosmidosine, as well as intramolecular methyl transfer reaction (c).

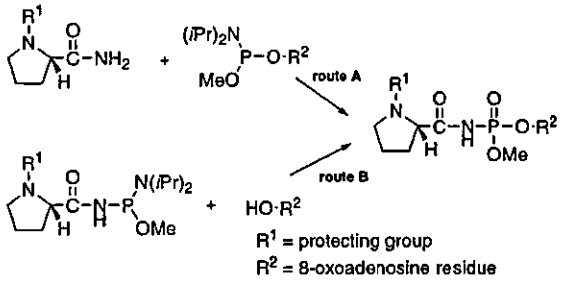

FIGURE 5. Two strategies for the synthesis of phosmidosine derivatives.

isopropylidene groups were used for the latter. There are two strategies for construction of the *N*-acyl phosphoramidate linkage, as shown in Figure 5. In our previous papers,[5,6] we reported the use of route A, since we observed that an intramolecular cyclization occurred when an *N*-(*N*-tritylphenylalanyl)phosphorodiamidite derivative was activated in the presence of 1*H*-tetrazole. However, this strategy gave the coupling product in only 27% yield.[6] Therefore, we reinvestigated route B again. In this type of condensation, van Boom reported that 5-mercapto-1-methyl-1*H*-tetrazole (MMT) was an excellent reagent.[10,11] Therefore, with the above-mentioned discussion in mind, we studied the synthesis of chemically stabilized phosmidosine analogues using MMT and acid-labile protecting groups.

Synthesis of 8-Oxoadenosine and an *N*-Phosphoramidite Derivative of Proline. In the synthesis of phosmidosine derivatives, 8-oxoadenosine **3** is a key intermediate. This compound was previously prepared by a two-step reaction from commercially available 8-bromoadenosine (**2**).[12] However, when the original procedure was employed, the total yield of **3** was only 42%. We found that the yield was dramatically improved to 84% when isolation of the intermediate, 6-*N*,2′,3′,5′-*O*-tetraacetyl-8-bromoadenosine, by the use of crystallization was omitted. Acetonization of **3** followed by the reaction with Boc_2O gave the 5′-unprotected product **4** in 73% yield.

The *N*-phosphoramidite building units **7a–d** were also synthesized by phosphitylation of an N-tritylated prolinamide derivative **5** with various alkyl *N*,*N*-bis(diisopropyl) phosphorodiamidite derivatives (**6a–d**).

Synthesis of a Fully Protected Phosmidosine Derivative. Condensation of **4** with **7a** in the presence of MMT followed by oxidation with *tert*-butyl hydroperoxide[13,14] gave the coupling product **8a** as a diastereomeric mixture in 66% yield. The previous method gave the same compound in 27% yield. Therefore, the present approach proved to be superior to the previous one. Actually, deprotection of this product gave a mixture of phosmidosine **1a-slow** and its diastereoisomer **1a-fast** in 69% yield, where the fast- and slow-eluting products in reverse-HPLC were named the "fast-eluted" and "slow-eluted" products **1a-fast** and **1a-slow**, respectively. Thus, the total yield of phosmidosine from 8-bromoadenosine was improved up to 23% compared with 2% resulting from the previous method. The diastereoisomers **1a-fast** and **1a-slow** were successfully isolated in 29 and 33% yields, respectively. The synthetic sample **1a-slow** was completely identified as the authentic sample obtained from a culture filtrate of *Streptomyces* sp. RK-16.[1]

Synthesis of Base-Resistant Phosmidosine Derivatives. To avoid the intramolecular and intermolecular methyl transfer reactions, we synthesized phosmidosine analogues **1b–d** replaced by more sterically hindered O-substituents. Compound **4** was similarly allowed to react with alkyl phosphorodiamidite derivatives **7b–d**, which were synthesized according to our previous method.[8]

It should be noted that, among the phosmidosine analogues **8b–d** thus obtained, compound **8b** could be synthesized in the highest yield of 95%, as shown in Table 1.

Particularly, in this case, the byproducts could be easily separated from the desired condensation product. Fur-

TABLE 1. Synthesis of Fully Protected Phosmidosine and Its Alkyl Ester Analogues 8a–d and Deprotection of 8a–d Giving Rise to Unprotected Phosmidosine Derivatives 1a–d

	condensation		deprotection			
compd	product	yield (%)	product	yield (%)	product	yield (%)
7a (R = Me)	**8a**	66	**1a-fast**	29	**1a-slow**	33
7b (R = Et)	**8b**	95	**1b-fast**	39	**1b-slow**	44
7c (R = *i*Pr)	**8c**	a	**1c-fast**	13	**1c-slow**	20
7d (R = Bu)	**8d**	a	**1d-fast**	6	**1d-slow**	7

[a] Coupling product was used in situ for the deprotection without isolation.

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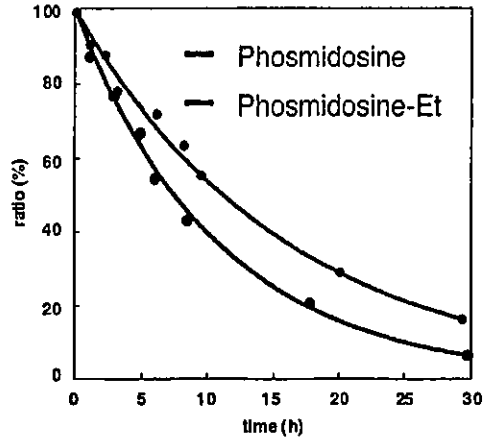


FIGURE 6. Stability of phosmidosine (black) and its *O*-ethyl ester analogue (red) in 10.1 M NaOH. The value on the *y*-axis is the percentage of the remaining sample.

TABLE 2. Antitumor Activities of Phosmidosine Analogues[a]

R of **1**	diastereomer	IC_{50} (μM) KB	IC_{50} (μM) L1210
Me	**1a-fast**	0.9	4.5
	1a-slow	0.6	1.9
Et	**1b-fast**	2.7	4.8
	1b-slow	1.2	5.6
*i*Pr	**1c-fast**	6.5	13.6
	1c-slow	4.0	5.5
Bu	**1d-fast**	3.1	13.0
	1d-slow	1.4	3.4

[a] Inhibition ratio was calculated by the following formula: (1 − treated OD/control OD) × 100.

thermore, treatment of **8b** with 80% formic acid gave a diastereomeric mixture of the ethyl esters **1b-fast** and **1b-slow**, which were found to be easily separated by medium-pressure C_{18} reverse-phase column chromatography and could be isolated in 39 and 44% yields, respectively. In the case of **1c** and **1d**, it was somewhat difficult to separate the diastereomers. In a 0.1 M NaOH solution, the phosmidosine ethyl ester analogues **1b-fast** and **1b-slow** were found to be 1.5 times more stable than phosmidosine **1a**, as shown in Figure 6.

Antitumor Activity of Phosmidosine Analogues. To examine the effects of the O-substituent and each diastereoisomer of the phosmidosine analogues **1b−d** on the antitumor activity compared with those of phosmidosine, we chose KB and L1210 cell lines. These results are summarized in Table 2.

As the general tendency, there is no significant difference between the two diastereoisomers of **1a−d**. Particularly, the ethyl ester **1b** maintained significant activities similar to those of phosmidosine **1a**. In consideration of the ease of the synthesis and the chemical stability of the ethyl ester, we decided to use **1b** as a core structure to study the structure−activity relationship of phosmidosine.

Effects of O-Substituted Phosmidosine Analogues on Morphological Reversion of v-src^{ts}NRK Cells. Phosmidosine has biological activity capable of morphological reversion of v-src^{ts}NRK cells, as reported previously. To compare the synthetic O-substituted

TABLE 3. Morphological Reversion Activity of O-Substituted Phosmidosine Analogues[a]

compd	morphological reversion acivity (μg/mL) 1	2	10	30	100	ED_{50}	cell cycle arrest ED100 (mg/mL)
1a	+	++	+++	+++	+++	3	10
1b	+	++	+++	+++	+++	3	10
1c	−	++	++	+++	+++	3	30
1d	−	++	++	+++	+++	3	30
1a-fast	+	++	+++	+++	+++	3	10
1a-slow	+	++	+++	+++	+++	3	10
1b-fast	+	++	+++	+++	+++	3	10
1b-slow	+	++	+++	+++	+++	3	10

[a] Symbol −: the state where all cells show round cancer cells. Symbol +: ca. 25% of cells are reversed to normal cells. Symbol ++: 25−75% of cells are reversed to normal cells. Symbol +++: more than 75% of cells are reversed to normal cells.

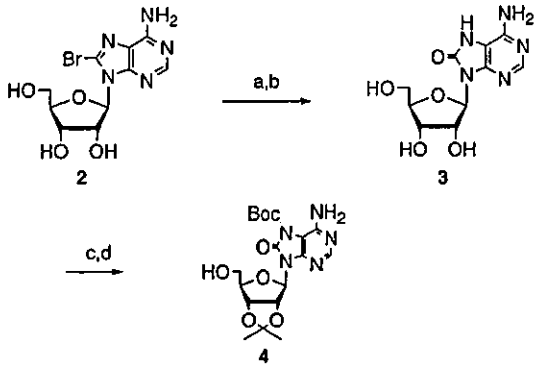
phosmidosine derivatives with phosmidosine, the diastereomeric mixtures of compounds **1a−d** were tested for this morphological reversion activity. These results are summarized in Table 3. The ED_{50} value refers to the concentration of a sample where 50% of v-src^{ts}NRK cells are reversed to normal cells. All compounds tested showed the same ED_{50} value of 3 μg/mL. The ED_{100} value means the concentration of a sample when the cell cycle is completely arrested at the G_1 phase. In the case of phosmidosine and the *O*-ethyl derivative, they showed high activity of ED_{100} 10 μg/mL. There is a tendency for the activity to decrease with an increase in the alkyl chain. Furthermore, each of the diastereomers of **1a** and **1b** was also tested for the same analysis. As a result, there is no distinct difference in the activity between the stereoisomers in both compounds. These results are almost in agreement with those obtained in the above-mentioned antitumor analysis using KB and L1210 cell lines.

Superiority of the Present Method in the Synthesis of Phosmidosine Ethyl Ester Derivative 8b. In our previous paper,[5,6] we reported the first synthesis of phosmidosine from *N*-trityl-L-prolinamide and *N*^{6}-*tert*-butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-(methyl *N*,*N*-diisoprpylphosphoramidite). In this synthesis, the P−N bond formation was carried out in the presence of 5-(3,5-dinitrophenyl)-1*H*-tetrazole (DNPT)[5,6] as the activator to give the coupling product in 27% yield. In a similar manner, an 8-oxoadenosine 5′-phosphoramidite derivative **9** was synthesized in 89% yield and activated by the same reagent to obtain the coupling product **8b**.

However, the desired product **8b** was obtained in a poorer yield. It was found that the trityl group was considerably eliminated during the reaction. In the previous study, we did not observe such a serious side reaction. This is due to the relatively high acidity of this reagent. Replacement of this reagent by 1*H*-tetrazole or diisopropylammonium 1*H*-tetrazolide[15] led to no reaction. The addition of pyridine or triethylamine to DNPT also failed. The best result was obtained when 1 equiv of DNPT to the phosphoramidite derivative was used. Thus, the coupling product **8b** was obtained in 27% yield. This is the same level as that of the previous synthesis of

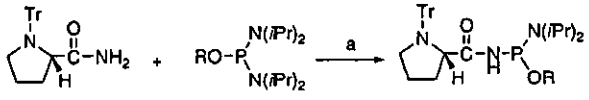
(15) Barone, A. D.; Tang, J.-Y.; Caruthers, M. H. *Nucleic Acids. Res.* **1984**, *12*, 4051−4061.

SCHEME 1 [a]



[a] Reagents: (a) NaOAc, AcOH–Ac_2O (1:1, v/v); (b) 0.1 M NaOH, EtOH (84%); (c) $Me_2C(OM)_2$, TsOH, acetone; (d) $(Boc)_2O$, MeOH–Et_3N (9:1, v/v) (73%).

SCHEME 2 [a]



a: R = Me, b: R = Et, c: R = iPr, d: R = Bu

[a] Reagents: (a) diisopropylammonium 1*H*-tetrazolide, CH_2Cl_2.

phosmidosine via route A shown in Figure 5. Therefore, the coupling mode using the *N*-trityl-L-prolylphosphorodiamidite derivative **7b** and **4** is superior to the above synthetic mode.

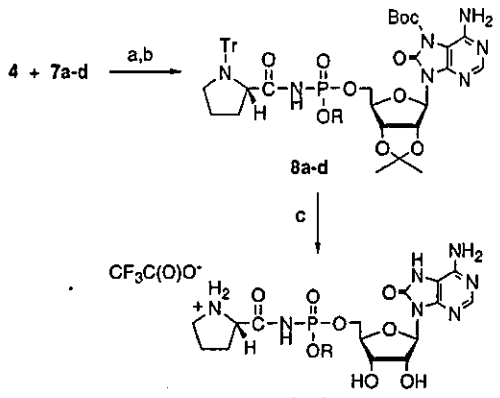
Structure–Activity Relationship of Phosmidosine: Synthesis of Phosmidosine Derivatives and Related Compounds Lacking Partial Structures. To understand which part of phosmidosine is important, we tried to synthesize diethyl *N*-acetylphosphoramidate, i.e., a core structure of phosmidosine without the proline and 8-oxoadenosine residues. It was reported that this compound could be obtained by the reaction of diethyl isocyanatophosphonate with acetic acid.[16] However, this reaction gave tetraethyl pyrophosphate as the main product. We also failed in other attempts involving the reaction of diethyl phosphoramidate with acetyl chloride or acetic anhydride and the reaction of acetamide with diethyl phosphorochloridate. The most effective method we found ultimately involves the use of phosphoramidite chemistry, as used in the synthesis of phosmidosine. Reaction of acetamide with diethyl *N,N*-diisopropylphosphoramidite (**10**) in the presence of 1*H*-tetrazole in acetonitrile followed by oxidation with *tert*-butyl hydroperoxide gave the desired compound **11** in 40% yield.

Next, an *N*-prolylphosphoramidate derivative **13** lacking the 8-oxoadenosine moiety was synthesized, as shown in Scheme 6. On the other hand, an 8-oxoadenosine *N*-acetylphosphoramidate derivative **15** was prepared by reaction of **9** with acetamide followed by acidic treatment of the resulting product **14**, as shown in Scheme 7.

The antitumor activities of these compounds are shown in Table 4. It is somewhat interesting that compound **11** showed weak cytotoxicities against KB and L1210. From

(16) Nikonorov, K. V.; Latypov, Z. Ya.; Antokhina, L. A. *Zh. Obsh. Khim.* **1982**, *52*, 2645–2646.

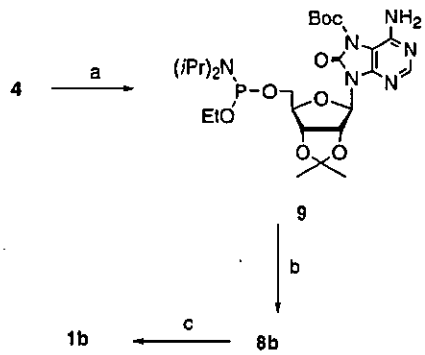
SCHEME 3 [a]



a: R = Me, b: R = Et, c: R = iPr, d: R = Bu

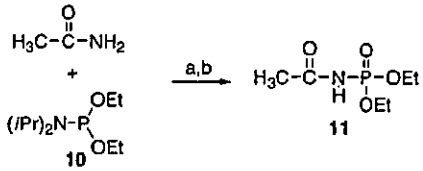
[a] Reagents: (a) MMT, CH_3CN; (b) *t*BuOOH; (c) 80% HCOOH.

SCHEME 4 [a]



[a] Reagents: (a) $EtOP[(N(iPr)_2]_2$, diisopropylammonium 1*H*-tetrazolide, CH_2Cl_2; (b) **5**, DNT, CH_3CN; (c) *t*BuOOH; (d) 80% HCOOH.

SCHEME 5 [a]



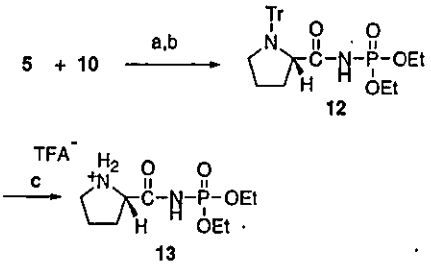
[a] Reagents: (a) 1*H*-tetrazole, CH_3CN; (b) *t*BuOOH.

the experiments using **13** and **15**, both the proline and 8-oxoadenosine residues are very important for the antitumor activity of phosmidosine.

Synthesis of a Phosmidosine Analogue Having an *N*-Prolylphosphoramidothioate Linkage. To examine the importance of the phosphoryl group, the phosphoramidothioate derivative **17** was also prepared as a diastereomeric mixture, as shown in Scheme 8. It was difficult to separate the diastereoisomers in this case. This compound was found to be very stable. The antitumor activities of this compound were essentially maintained, as shown in Table 5.

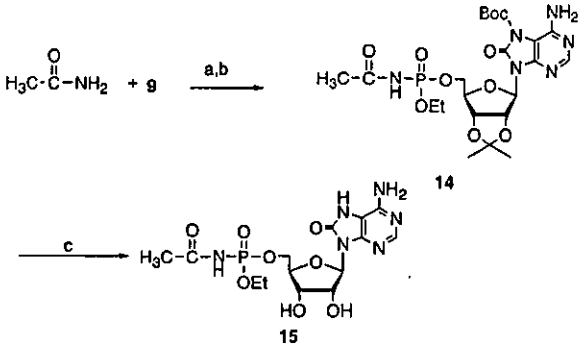
Effect of Enantiomer of the Amino Acid Component on the Antitumor Activity. To study the effect of the steric environment around the amino acid residue on the antitumor activity of phosmidosine, we changed

SCHEME 6 [a]



[a] Reagents: (a) 1*H*-tetrazole, CH_3CN; (b) *t*BuOOH; (c) TFA.

SCHEME 7 [a]



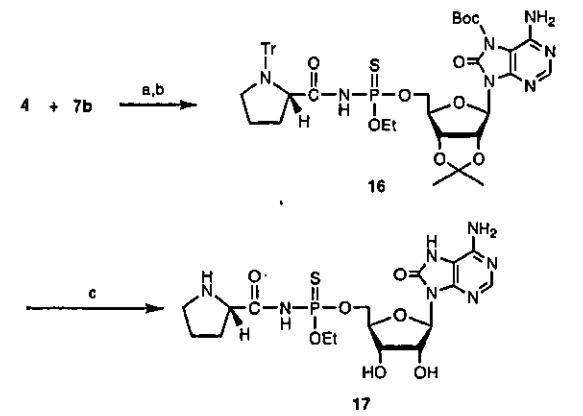
[a] Reagents: (a) 1*H*-tetrazole, CH_3CN; (b) *t*BuOOH; (c) TFA.

TABLE 4. Antitumor Activities of Phosmidosine Analogues Lacking Partial Structures

	IC_{50} (μM)[a]	
compd	KB	L1210
1b-fast,slow	1.1	1.6
11	>80	>80
13	>80	>80
15	>80	>80

[a] Phosmidosine analogues with IC_{50} values over 80 μM showed the inhibitory effects under 20% at the concentration of 80 μM.

SCHEME 8 [a]



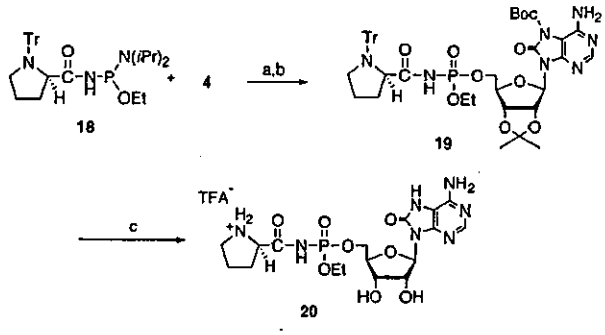
[a] Reagents: (a) MMT, CH_3CN; (b) $[Et_2NC(S)S]_2$; (c) TFA.

the L-proline residue to D-proline. The synthesis of this compound was similarly conducted using the corresponding *N*-trityl-D-prolylphosphorodiamidite derivative **18**, as shown in Scheme 9.

TABLE 5. Antitumor Activities of Phosmidosine Phosphoramidothioate

	IC_{50} (μM)	
compd	KB	L1210
1b-fast,slow	3.4	3.6
17	2.7	15.0

SCHEME 9 [a]



[a] Reagents: (a) MMT, CH_3CN; (b) *t*BuOOH; (c) 80% HCOOH.

TABLE 6. Antitumor Activities of Phosmidosine Analogue Having a D-Proline Residue

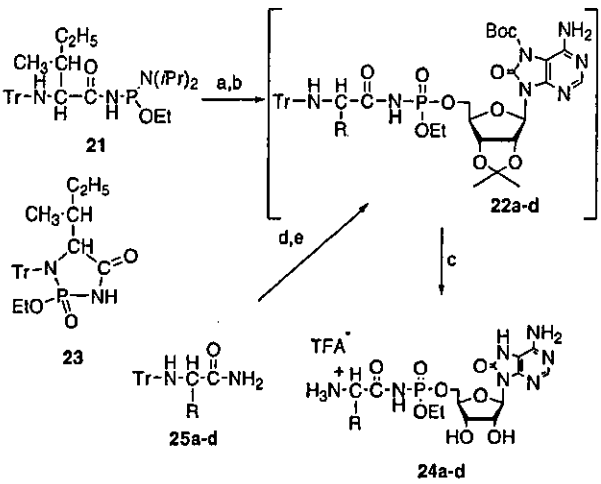
	IC_{50} (μM)	
compd	KB	L1210
1b-fast,slow (L-deriv)	1.1	1.6
20 (D-deriv)	29.2	51.8

The antitumor activity of the phosmidosine analogues having D- and L-prolines is shown in Table 6. Interestingly, the D-isomer showed markedly decreased IC_{50} values in KB and L1210 cell lines compared with those of the phosmidosine ethyl ester derivative. In these assays, diastereomeric mixtures due to the chirality of the phosphorus center were used.

Effects of Other Amino Acids on the Antitumor Activity. To see if replacement of the proline moiety by other amino acid residues affects the antitumor activity, several phosmidosine-related derivatives **22a–d** were synthesized, as shown in Scheme 10.

However, reaction of an *N*-trityl-L-isoleucylphosphorodiamidite derivative **21** with **4** in the presence of MMT followed by the in situ treatment with 80% formic acid gave the desired product **22a** in only 4% yield. In this reaction, it was found that **23**, an intramolecularly cyclized product of **21**, was predominantly formed. This type of side reaction was also reported by us when we tried the condensation of methyl *N*-trityl-L-phenylalanylphosphorodiamidite with 2′,3′-*O*,6-*N*-tribenzoyladenosine in the presence of 1*H*-tetrazole.[6] Actually, this undesired side reaction led us to study an alternative route to phosmidosine so that, in our previous paper, we employed route A of Figure 5. However, as mentioned above, in the case of the *N*-prolylphosphorodiamidite derivative, it underwent smooth condensation with **4** in the presence of MMT. This outcome is explained in terms of the difference in steric hindrance between the secondary amine of proline and the primary amine of phenylalanine or isoleucine. It was concluded that the strategy depicted via route B in Figure 5 is only available for compounds having the secondary amino group, while

SCHEME 10 [a]



a : R = -CH(CH$_3$)(CH$_2$CH$_3$) (L-deriv), **b**: R = -CH(CH$_3$)(CH$_2$CH$_3$) (D-deriv);**c**: R = CH$_3$ (L-deriv); **d**: R = -CH$_2$CH$_2$SCH$_3$ (L-deriv)

[a] Reagents: (a) **4**, MMT, CH$_3$CN; (b) *t*BuOOH; (c) 80% HCOOH; (d) **9**, DNPT, CH$_3$CN; (e) 1 M I$_2$, pyridine–H$_2$O (9:1, v/v).

TABLE 7. Antitumor Activities of Phosmidosine Analogues Replaced by Other Amino Acid Residues

compd	IC$_{50}$ (μM)[a] KB	L1210
1b-fast,slow	1.1	1.6
24a	>80	>80
24b	>80	>80
24c	>80	>80
24d-fast	>80	>80
24d-slow	>80	>80

[a] Phosmidosine analogues with IC$_{50}$ values over 80 μM showed inhibitory effects under 20% at a concentration of 80 μM.

route B is suitable for proline derivatives. Therefore, for the synthesis of compounds **22a–d** having L-isoleucine, D-isoleucine, L-alanine, and L-methionine, our previous strategy involving the activation of adenosine 5′-phosphoramidite derivatives via route A is actually better. Thus, these modified analogues could be synthesized and tested for antitumor activity. The results are shown in Table 7. Surprisingly, replacement of the proline residue with other amino acid residues resulted in a marked decrease in the biological activity.

Conclusion

On the basis of the results from the above experiments, the following conclusions were reached. (1) The methyl group of the phosphoramidate linkage can be replaced by longer alkyl groups without significant decrease in the antitumor activity. (2) The proline residue and 8-oxoadenosine residue are both required for the biological expression. (3) Replacement of the proline moiety with other amino acid residues resulted in a marked loss of antitumor activity. Since aminoacyl adenylate analogues such as adenosine 5′-(*N*-aminoacyl)sulfonamide derivatives are known to inhibit peptide synthesis,[17–20] phosmidosine derivatives are expected to have similar inhibitory ability. If phosmidosine affects the peptide synthesis that is related to expression of the growth of tumor cells, phosmidosine analogues replaced by other amino acids should have similar activity. However, our results are not in agreement with this expectation. Otherwise, it is likely that phosmidosine analogues replaced with amino acids having primary amines tend to decompose when incorporated into cells. Actually, the isolated yields of these modified analogues are rather low. In the case of phosmidosine, N–N rearrangement is known to occur, as depicted in path b of Figure 4.[2] Therefore, these modified analogues having the primary amine undergo more rapid N–N rearrangement in cells to lose their biological activity. Thus, the possibility that phosmidosine and its derivatives synthesized in this study affect the peptide synthesis as inhibitors cannot be ruled out. It is likely that only the proline derivative can survive in nature, allowing phosmidosine to be discovered.

Experimental Section

^{31}P NMR Analysis of Phosmidosine at Various pHs. A diastereomeric mixture of phosmidosine methyl esters **1a-fast** and **1b-slow** was dissolved in 200 μL of an appropriate 1 M citric–citrate buffer at pH 3, 4, 5, 6, and 7 so as to obtain a 40 mM solution of phosmidosine. After being kept at room temperature for 10 min, the solution was analyzed by use of 85% H$_3$PO$_4$ as the external reference.

8-Oxoadenosine (3). To a solution of 8-bromoadenosine (2) (10.3 g, 30 mmol) in acetic acid–acetic anhydride (1:1, v/v, 600 mL) was added sodium acetate (45 g, 549 mmol). After being stirred at 120 °C for 3 h, the mixture was diluted with ethyl acetate. The solution was washed five times with water, and the organic layer was collected, dried over Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was dissolved in ethyl acetate, and this solution was washed three times with 5% NaHCO$_3$ and evaporated under reduced pressure. The residue was dissolved in EtOH (600 mL), and NaOH (24 g, 600 mmol) was added. After being stirred at 60 °C for 3 h, the mixture was neutralized by addition of 4 M HCl (100 mL) followed by addition of 5% NaHCO$_3$. The precipitates were removed by filtration and washed three times with water. The filtrate and washing were collected and evaporated under reduced pressure. Trituration of the amorphous material with water–*i*PrOH (10:1, v/v, 20 mL) followed by collection by filtration gave **3** as a white solid (7.1 g, 84%): ^{1}H NMR (270 MHz, DMSO) δ 3.43–3.56 (2H, m), 3.79 (1H, bs), 4.05 (1H, bs), 4.76–4.82 (1H, m), 4.99–5.00 (1H, m), 5.09–5.13 (1H, m), 5.17–5.19 (1H, m), 5.60 (1H, d, J = 2.0 Hz), 6.49 (2H, bs), 7.94 (1H, s), 10.30 (1H, bs); ^{13}C NMR (CDCl$_3$) δ 62.4, 70.3, 71.0, 85.4, 85.7, 103.5, 156.4, 147.0, 150.5, 151.4; ESI-mass *m/z* calcd for C$_{10}$H$_{14}$N$_5$O$_5$ 284.0995, observed [M + H] 284.0997.

***N*^{2}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine (4).** To a suspension of 8-oxoadenosine (**3**) (5.10 g, 18 mmol) in acetone (180 mL) were added 2,2-dimethoxypropane (44.3 mL, 360 mmol) and *p*-toluenesulfonic acid monohydrate (6.85 g, 36 mmol). After being stirred at room temperature for 4 h, the mixture was quenched by addition of saturated NaHCO$_3$. The mixture was evaporated under reduced pressure. The residue was partitioned between CHCl$_3$–*i*PrOH (3:1, v/v) and 5% NaHCO$_3$. The organic layer was collected, dried over Na$_2$SO$_4$, filtered, and evaporated under reduced pressure. The residue was dissolved in MeOH–Et$_3$N

(17) Ubukata, M.; Isono, K. *Tetrahedron Lett.* **1986**, *27*, 3907–3908.
(18) Castro-Pichel, J.; Garcia-Lopez, M. T.; De las Heras, F. G. *Tetrahedron* **1987**, *43*, 383–389.
(19) Ubukata, M.; Osada, H.; Magae, J.; Isono, K. *Agr. Biol. Chem.* **1988**, *52*, 1117–1122.
(20) Landeka, I.; Filipic-Rocak, S.; Zinic, B.; Weygand-Durasevic, I. *Biochim. Biophys. Acta* **2000**, *1480*, 160–170.

(9:1, v/v, 200 mL), and di-*tert*-butyl dicarbonate was added. After being stirred at room temperature for 2 h, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$, and the organic layer was collected, dried Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (from 100:0 to 97:3, v/v) to give **4** (5.58 g, 73%): ^{1}H NMR (270 MHz, DMSO) δ 1.29 (3H, s), 1.49 (3H, s), 1.56 (9H, s), 3.46–3.58 (2H, m), 4.04–4.09 (1H, m), 4.87–4.91 (2H, m, J = 3.3 Hz), 5.36 (1H, dd, J = 6.3 Hz), 5.92 (1H, d, J = 2.3 Hz), 7.03 (2H, bs), 8.11 (1H, s); ^{13}C NMR ($CDCl_3$) δ 25.5, 27.7, 28.0, 63.4, 81.2, 81.3, 85.2, 87.0, 89.1, 102.1, 113.9, 147.1, 147.9, 149.0, 149.8, 153.1; ESI-mass *m/z* calcd for $C_{16}H_{26}N_5O_7$ 424.1832, observed [M + H] 424.1734.

General Procedure for the Synthesis of Alkyl *N,N*-Diisopropyl-*N*′-[*N*-trityl-L-prolyl]phosphorodiamidites 7a–d. A mixture of *N*-trityl-L-prolinamide (**5**) (107 mg, 0.30 mmol) and *N,N*-diisopropylammonium 1*H*-tetrazolide (31 mg, 0.18 mmol) was rendered anhydrous by coevaporation three times with anhydrous toluene and finally dissolved in dry CH_2Cl_2 (3 mL). To the solution was added methyl *N,N,N′,N′*-tetraisopropylphosphorodiamidite (94 μL, 0.39 mmol). After being stirred at room temperature for 4 h, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc–Et_3N (from 100:0:1 to 90:10:1, v/v/v) to give **7a** (138 mg, 89%): ^{1}H NMR (270 MHz, DMSO) δ 1.17–1.90 (14H, m), 1.65–1.70 (1H, m), 3.00–3.01 (1H, m), 3.29–3.33 (1H, m), 3.74–4.34 (6H, m), 7.69–7.81 (9H, m), 7.97–8.07 (6H, m), 8.38 (1H, 2bs); ^{13}C NMR (DMSO) δ 23.9, 24.1, 24.2, 24.3, 24.4, 24.5, 31.0, 43.3, 44.5, 49.9, 51.2, 51.4, 51.5, 51.7, 63.9, 64.7, 77.4, 77.5, 126.0, 126.1, 127.5, 127.6, 128.7, 144.4, 144.6, 177.1, 177.3, 177.5, 177.7; ^{31}P NMR (DMSO) δ 117.77, 118.50; ESI-mass *m/z* calcd for $C_{31}H_{41}N_3O_2P$ 518.2936, observed [M + H] 518.2866.

Compounds **7b–d** were similarly synthesized in 83, 91, and 78% yields, respectively, but elution for silica gel column chromatography was performed with hexanes–EtOAc–Et_3N using (100:0:1–95:5:1, 100:0:1–92:8:1, and 100:0:1–85:15:1, respectively, v/v/v).

7b: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.81–1.1.47 (18H, m), 1.65–1.70 (1H, m), 2.97–3.04 (1H, m), 3.23–3.27 (1H, m), 3.63–3.87 (5H, m), 7.13–7.26 (9H, m), 7.50–7.53 (6H, m), 8.07 (1H, 2bs); ^{13}C NMR ($CDCl_3$) δ 18.6, 18.7, 18.8, 18.8, 25.7, 25.8, 25.9, 26.0, 26.1, 26.1, 32.5, 32.6, 45.6, 45.8, 45.9, 46.1, 51.9, 56.6, 62.2, 62.5, 67.1, 67.2, 127.7, 129.1, 130.5, 130.6, 145.9, 146.1, 179.7, 179.8; ^{31}P NMR ($CDCl_3$) δ 112.96, 114.49; ESI-mass *m/z* calcd for $C_{32}H_{43}N_3O_2P$ 532.3093, observed [M + H] 532.3030.

7c: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.77–1.46 (21H, m), 1.64–1.77 (1H, m), 2.94–3.09 (1H, m), 3.19–3.32 (1H, m), 3.60–3.79 (2H, m), 3.85–3.88 (1H, m), 4.21–4.31 (1H, m), 7.13–7.26 (9H, m), 7.50–7.53 (6H, m), 8.07 (1H, 2bs); ^{13}C NMR ($CDCl_3$) δ 24.3, 24.4, 24.5, 24.6, 24.7, 31.0, 31.1, 44.2, 44.4, 44.5, 44.7, 50.4, 65.6, 65.7, 68.4, 68.5, 68.8, 69.0, 78.2, 78.3, 126.3, 127.6, 127.8, 129.1, 144.5, 144.6, 178.0, 178.2, 178.4; ^{31}P NMR ($CDCl_3$) δ 110.97, 112.69; ESI-mass *m/z* calcd for $C_{33}H_{45}N_3O_2P$ 546.3249, observed [M + H] 546.3294.

7d: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.76–1.16 (5H, m, J = 7.3 Hz), 1.21–1.55 (15H, m), 1.61–1.81 (3H, m), 2.97–3.12–3.09 (1H, m), 3.22–3.34 (1H, m), 3.62–3.91 (5H, m), 7.13–7.27 (9H, m, Ar–H), 7.51–7.55 (6H, m), 8.11 (1H, 2bs, CONH); ^{13}C NMR ($CDCl_3$) δ 13.7, 13.8, 19.0, 19.1, 24.0, 24.4, 24.5, 30.9, 31.1, 33.3, 33.4, 44.1, 44.3, 44.4, 50.3, 64.5, 64.5, 64.8, 64.9, 65.5, 65.6, 77.2, 78.1, 78.1, 126.1, 127.5, 128.9, 129.0, 144.3, 144.5, 178.0, 178.2, 178.2, 178.4; ^{31}P NMR ($CDCl_3$) δ 113.38, 114.90; ESI-mass *m/z* calcd for $C_{34}H_{47}N_3O_2P$ 560.3406, observed [M + H] 560.3441.

***N*⁶-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Methyl *N*-(*N*-Trityl-L-prolyl)phosphoramidate] (8a).** A mixture of **4** (847 mg, 2.0 mmol) and **7a** (2.07 g, 4.0 mmol) was coevaporated four times with dry acetonitrile and finally dissolved in dry acetonitrile (30 mL). To the mixture was added MMT (581 mg, 5.0 mmol), and the solution was stirred at room temperature for 1 h; then, a 6 M solution of *tert*-butyl hydroperoxide in decane (3.34 mL, 20.0 mmol) was added. After being stirred at room temperature for an additional 10 min, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc-pyridine, 50:50:1–40:60:1, v/v/v) to give a diastereomeric mixture of **8a** (1,12 g, 66%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.71–0.73 (1H, m), 1.04–1.41 (6H, m), 1.45 (1H, 2s, CH_3 of isop), 1.52 (9H, s), 2.87–2.90 (1H, m), 3.23–3.25 (1H), 3.80 (3H, 2d, $J_{P,H}$ = 11.9 Hz), 3.81–3.91 (1H, m), 4.31–4.37 (3H, m), 5.00–5.01 (1H, m), 5.34 (1H, dd, $J_{2',3'}$ = 6.3 Hz), 6.15 (1H, 2d, $J_{1',2'}$ = 1.3 Hz), 6.55 (2H, bs), 7.02–7.21 (9H, m), 7.32–7.44 (6H, m), 8.04 (1H, 2s); ^{13}C NMR ($CDCl_3$) δ 21.5, 24.3, 24.4, 25.5, 27.2, 28.0, 31.7, 31.8, 50.7, 50.8, 54.3, 54.4, 54.4, 54.5, 54.5, 65.6, 65.7, 67.3, 67.4, 78.3, 81.3, 81.8, 82.0, 82.8, 83.0, 85.2, 85.6, 85.7, 85.8, 86.7, 86.7, 87.0, 87.1, 102.0, 113.9, 114.0, 125.2, 126.6, 127.3, 127.9, 128.1, 128.9, 129.1, 129.1, 143.9, 147.6, 147.6, 148.1, 148.7, 148.8, 149.8, 153.6, 166.5, 177.4, 177.4; ^{31}P NMR ($CDCl_3$) δ −0.37, −0.46; ESI-mass *m/z* calcd for $C_{43}H_{51}N_7O_{10}P$ 856.3435, observed [M + H] 856.3437.

***N*⁶-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N*-(*N*-Trityl-L-prolyl)phosphoramidate] (8b): Method A.** A mixture of **4** (805 mg, 1.9 mmol) and **7b** (2.09 g, 3.6 mmol) was coevaporated four times with dry acetonitrile and finally dissolved in dry acetonitrile (30 mL). To the mixture was added MMT (552 mg, 4.75 mmol), and the solution was stirred at room temperature for 1 h; then, a 6 M solution of *tert*-butyl hydroperoxide in decane (3.2 mL, 19.0 mmol) was added. After being stirred at room temperature for an additional 10 min, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc–pyridine (50:50:1–40:60:1, v/v/v) to give a diastereomeric mixture of **8b** (1,57 g, 95%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.81–0.88 (1H, m), 1.07–1.50 (9H, m), 1.54–1.57 (1H, 2s), 1.62 (9H, s), 2.96–3.03 (1H, m), 3.31–3.36 (1H, m), 3.89–3.94 (2H, m), 4.19–4.50 (4H, m), 5.08–5.13 (1H, m), 5.43–5.45 (1H, m), 6.23–6.26 (1H, m), 6.62 (2H, bs), 7.13–7.32 (9H, m,), 7.45–7.67 (6H, m), 8.15–8.16 (1H, 2s); ^{13}C NMR ($CDCl_3$) δ 15.9, 16.0, 16.0 16.1, 24.1, 24.1, 24.3, 27.0, 27.8, 31.0, 31.4, 31.4, 50.2, 50.4, 50.5, 63.9, 64.0, 64.0, 64.1, 64.8, 65,3, 65.3, 67.0, 77.2, 78.0, 78.1, 81.6, 81.8, 82.6, 82.7, 85.4, 85.5, 85.6, 86.3, 86.3, 86.9, 86.9, 101.7, 101.7, 113.65, 113.7, 126.1, 126.2, 127.5, 127.6, 128.9, 142.9, 143.8, 144.3, 147.2, 147.3, 147.8, 148.7, 148.7, 149.5, 149.6, 153.3, 177.2, 177.2, 177.3, 177.3; ^{31}P NMR ($CDCl_3$) δ −1.82; ESI-mass *m/z* calcd for $C_{44}H_{53}N_7O_{10}P$ 870.3592, observed [M + H] 870.4179. **Method B.** A mixture of **9** (42.5 mg, 0.075 mmol) and **5** (17.9 mg, 0.050 mmol) was coevaporated three times with dry acetonitrile and finally dissolved in dry acetonitrile (10 mL). To the solution was added DNPT (11.9 mg, 0.050 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 1 h. A 6 M solution of *tert*-butyl hydroperoxide in decane (41.9 mL, 0.252 mmol) was added, and additional stirring was continued at room temperature for 10 min. The solution was diluted with $CHCl_3$, and the $CHCl_3$ solution was washed three times with 5% $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc–pyridine (50:50:1, v/v/v) to give **8b** (11.8 mg, 27%):

Diastereomers of Phosmidosine (1a). Compound **8a** (1.12 g, 1.31 mmol) was dissolved in 80% formic acid (15 mL). After being stirred at room temperature for 12 h, the mixture

was diluted with distilled water. The aqueous solution was washed 3 times with EtOAc, evaporated under reduced pressure, and coevaporated three times with distilled water. The residue was chromatographed on a column of reverse-phase C_{18} silica gel with water–acetonitrile (100:0–95:5, v/v) to give the fraction containing **1a**. Evaporation of this fraction under reduced pressure followed by lyophilization gave a diastereomeric mixture of **1a** (425 mg, 69%). Further medium-pressure C_{18} reverse-phase column chromatography with solvent system III gave **1a-fast** (179 mg, 29%) and **1a-slow** (204 mg, 33%). **1a-fast:** ^{1}H NMR (270 MHz, D_2O) δ 1.91–2.05 (3H, m), 2.30–2.36 (1H, m), 3.26–3.41 (2H, m), 3.55 (3H, d), 4.10–4.22 (4H, m), 4.60 (1H, m, 3′-H), 5.14 (1H, dd, $J_{2',3'}$ = 5.6 Hz), 5.87 (1H, d, $J_{1',2'}$ = 4.0 Hz), 8.09 (1H, s); ^{13}C NMR (D_2O) δ 26.3, 32.2, 48.9, 56.2, 56.3, 64.0, 64.4, 68.5, 68.6, 73.1, 73.2, 84.4, 84.2, 84.3, 88.8, 106.7, 149.0, 149.8, 153.6, 155.1, 177.0; ^{31}P NMR (D_2O) δ −1.42; ESI-mass *m/z* calcd for $C_{16}H_{25}N_7O_8P$ 474.1502, observed [M + H] 474.1501. **1a-slow:** ^{1}H NMR (270 MHz, D_2O) δ 1.90–2.05 (3H, m), 2.27–2.36 (1H, m), 3.26–3.50 (2H, m), 3.53 (3H, d, J_{POCH} =11.1 Hz), 4.08–4.21 (4H, m), 4.60 (1H, m), 5.16 (1H, dd, $J_{2',3'}$ = 5.5 Hz), 5.87 (1H, d, $J_{1',2'}$ = 4.6 Hz), 8.10 (1H, s); ^{13}C NMR (D_2O) δ 26.4, 32.4, 48.9, 55.8, 64.6, 64.9, 68.1, 72.34, 73.2, 84.4, 84.5, 88.8, 106.3, 148.8, 149.4, 153.5, 154.9, 178.3; ^{31}P NMR (D_2O) δ −1.32; ESI-mass *m/z* calcd for $C_{16}H_{25}N_7O_8P$ 474.1502, observed [M + H] 474.1501.

Diastereomers of Phosmidosine Ethyl Ester 1b. Compound **8b** (1.57 g, 1.81 mmol) was dissolved in 80% formic acid (20 mL). After the mixture was stirred at room temperature for 12 h, the same workup as described above gave a diastereomeric mixture of **1b** (733 mg, 83%). Further medium-pressure reverse-phase column chromatography with solvent system II gave **1b-fast** (335 mg, 39%) and **1b-slow** (388 mg, 44%). **1b-fast:** ^{1}H NMR (270 MHz, D_2O) δ 1.23–1.28 (3H, t, J = 7.3 Hz), 1.96–2.13 (3H, m), 2.42–2.52 (1H, m), 3.34–3.47 (2H, m), 4.10–4.25 (3H, m, $J_{P,H}$ = 8.9 Hz), 4.31–4.47 (3H, m), 4.65 (1H, m), 5.04 (1H, dd, $J_{2',3'}$ = 5.6 Hz), 5.93 (1H, d, $J_{1',2'}$ = 4.0 Hz), 8.34 (1H, s); ^{13}C NMR (D_2O) δ 17.9, 18.0, 26.2, 32.0, 49.1, 63.0, 63.2, 68.4, 68.5, 69.6, 69.7, 72.2, 73.8, 84.3, 84.4, 89.3, 107.1, 112.4, 116.7, 121.0, 125.3, 144.6, 146.8, 149.0, 154.8, 164.4, 165.0, 165.5, 166.0, 173.9, 173.9; ^{31}P NMR (D_2O) δ −1.35; ESI-mass *m/z* calcd for $C_{17}H_{27}N_7O_8P$ 488.1659, observed [M + H] 488.1666. **1b-slow:** ^{1}H NMR (270 MHz, D_2O) δ 1.24–1.29 (3H, t, J = 7.3 Hz) 2.01–2.22 (3H, m), 2.44–2.57 (1H, m), 3.35–3.51 (2H, m), 4.11–4.22 (2H, m, $J_{P,H}$ = 8.3 Hz), 4.27–4.30 (1H, m), 4.39–4.52 (3H, m), 4.67 (1H, m), 5.10 (1H, dd, $J_{2',3'}$ = 5.6 Hz), 5.97 (1H, d, $J_{1',2'}$ = 4.3 Hz), 8.38 (1H, s,); ^{13}C NMR (D_2O) δ 17.8, 17.9, 26.1, 31.9, 49.1, 63.0, 63.2, 68.4, 68.5, 69.7, 69.8, 72.2, 73.7, 84.3, 84.4, 89.2, 107.0, 112.5, 116.8, 121.1, 125.4, 144.6, 146.8, 149.0, 154.8, 164.5, 165.0, 165.5, 166.1, 173.9, 173.9; ^{31}P NMR (D_2O) δ −1.40; ESI-mass *m/z* calcd for $C_{17}H_{27}N_7O_8P$ 488.1659, observed [M + H] 488.1661.

Diastereomers of Phosmidosine Isopropyl Ester 1c. A mixture of **4** (580 mg, 1.4 mmol) and **7c** (1.50 g, 2.7 mmol) was coevaporated four times with dry acetonitrile and finally dissolved in dry acetonitrile (20 mL). To the mixture was added MMT (398 mg, 3.4 mmol), and the solution was stirred at room temperature for 1 h; then, a 6 M solution of *tert*-butyl hydroperoxide in decane (3.2 mL, 19.0 mmol) was added. After being stirred at room temperature for an additional 10 min, the same workup as that described in the case of **1b** gave the diastereomeric coupling product **8c**. This mixture was dissolved in 80% formic acid (10 mL), and the solution was stirred at room temperature for 12 h. A similar workup gave a diastereomeric mixture of **1c** (232 mg, 34%). Further medium-pressure reverse-phase column chromatography with solvent system II gave **1c-fast** (91 mg, 13%) and **1c-slow** (141 mg, 20%) as trifluoroacetate salts. **1c-fast:** ^{1}H NMR (400 MHz, D_2O) δ 1.05–1.07 (6H, 2d, J = 6.0 Hz), 1.77–1.89 (3H, m), 2.23–2.29 (1H, m), 3.15–3.27 (2H, m), 4.02–4.03 (1H, m), 4.12–4.27 (3H, m), 4.46–4.56 (2H, m, 3′-H), 4.87 (1H, m, $J_{2',3'}$ = 5.3 Hz), 5.72 (1H, d, $J_{1',2'}$ = 3.7 Hz), 8.09 (1H, s); ^{13}C NMR (D_2O) δ 25.2, 25.3, 25.4, 25.4, 26.1, 31.9, 49.1, 63.0, 63.2, 69.5, 69.6, 72.1, 73.7, 78.6, 78.7, 84.1, 84.2, 89.2, 106.9, 112.4, 116.7, 121.0, 125.3, 146.0, 148.7, 149.0, 154.9, 164.5, 165.0, 165.6, 166.1, 173.8, 173.8; ^{31}P NMR (D_2O) δ −2.71; ESI-mass *m/z* calcd for $C_{18}H_{29}N_7O_8P$ 502.1815, observed [M + H] 502.1854. **1c-slow:** ^{1}H NMR (270 MHz, D_2O) δ 1.25–1.27 (6H, 2d), 2.01–2.06 (3H, m), 2.45–2.50 (1H, m), 3.41–3.43 (2H, m), 4.24–4.25 (1H, m), 4.31–4.47 (3H, m), 4.64–4.73 (2H, m), 5.08–5.12 (1H, m), 5.92 (1H, d, $J_{1',2'}$ = 3.6 Hz), 8.31 (1H, s); ^{13}C NMR (D_2O) δ 25.2, 25.3, 25.3, 25.4, 26.1, 31.9, 49.1, 63.0, 63.2, 69.5, 69.6, 72.2, 73.6, 78.7, 78.7, 84.2, 84.3, 89.2, 106.9, 112.5, 116.8, 121.1, 125.4, 145.9, 148.6, 149.0, 154.9, 164.6, 165.1, 165.6, 166.1, 173.8, 173.8; ^{31}P NMR (D_2O) δ −2.80; ESI-mass *m/z* calcd for $C_{18}H_{29}N_7O_8P$ 502.1815, observed [M + H] 502.1854.

Diastereomers of Phosmidosine Butyl Ester 1d. This material was synthesized from **4** (953 mg, 2.3 mmol) and **7d** (2.62 g, 4.5 mmol) as described in the above experiment. **1d-fast:** ^{1}H NMR (270 MHz, D_2O) δ 0.79 (3H, t, J = 7.3 Hz), 1.16–1.27 (2H, m, J = 7.3 Hz), 1.48–1.55 (2H, m, J = 6.9 Hz), 4.21 (1H, m, 2″-H), 4.38–4.45 (3H, m), 4.64–4.66 (1H, m), 5.05–5.08 (1H, m, $J_{2',3'}$ = 5.3 Hz), 5.90 (1H, d, $J_{1',2'}$ = 3.6 Hz), 8.24 (1H, s); ^{13}C NMR (D_2O) δ 15.3, 20.6, 26.1, 32.0, 34.0, 34.1, 49.1, 63.0, 63.2, 69.6, 69.7, 71.8, 71.9, 72.0, 73.7, 84.0, 84.1, 89.2, 106.9, 112.5, 116.8, 121.0, 125.3, 146.8, 149.0, 149.8, 154.9, 164.6, 165.1, 165.7, 166.2, 173.9, 173,9; ^{31}P NMR (D_2O) δ −1.15; ESI-mass *m/z* calcd for $C_{19}H_{31}N_7O_8P$ 516.1972, observed [M + H] 516.2101. **1d-slow:** ^{1}H NMR (270 MHz, D_2O) δ 0.78–0.84 (3H, t, J = 7.3 Hz), 1.23–1.28 (2H, m), 1.52–1.54 (2H, m), 2.08 (3H, m), 2.49 (1H, m), 3.43 (2H, m), 4.05–4.08 (2H, m, J_{POCH} = 7.3 Hz), 4.26 (1H, m), 4.40–4.49 (3H, m), 4.65–4.69 (1H, m), 5.11–5.12 (1H, m), 5.92 (1H, d, $J_{1',2'}$ = 3.3 Hz), 8.32 (1H, s); ^{13}C NMR (D_2O) δ 15.3, 20.6, 26.1, 32.0, 33.9, 34.0, 49.1, 63.0, 63.2, 69.8, 69.8, 71.8, 71.9, 72.2, 73.6, 84.2, 84.4, 89.1, 106.9, 112.6, 116.9, 121.1, 125.4, 146.1, 148.9, 149.0, 154.9, 164.6, 165.1, 165.6, 166.2, 173.9, 173.9; ^{31}P NMR (D_2O) δ −1.35; ESI-mass *m/z* calcd for $C_{19}H_{31}N_7O_8P$ 516.1972, observed [M + H] 516.2101.

Stability of Phosmidosine 1a and Phosmidosine Et-Ester 1b. A sample was dissolved in 0.1 M NaOH to obtain a 0.111 mM solution of **1a** or **1b**. In this experiment, a diastereomeric mixture of **1a** or **1b** was used. The rate of decomposition of these materials was analyzed by reverse-phase HPLC.

***N*^{2}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N,N*-Diisopropylphosphoramidite] (9).** A mixture of **4** (423 mg, 1.0 mmol) and *N,N*-diisopropylammmonium 1*H*-tetrazolide (103 mg, 0.6 mmol) was rendered anhydrous by coevaporation three times with dry pyridine and dry toluene and finally dissolved in dry CH_2Cl_2 (10 mL). To the mixture was added ethyl (*N,N,N′,N′*-tetraisopropyl)phosphorodiamidite (310 μL, 1.1 mmol). After being stirred under argon an atmosphere at room temperature for 2 h, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc–Et_3N (95:5:1, v/v/v) to give **9** (531 mg, 89%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.93–1.21 (15H, m), 1.23 (3H, s), 1.44 (3H, s), 1.51 (9H, s), 3.31–3.67 (6H, m), 4.19–4.25 (1H, m), 4.89–4.92 (1H, m, $J_{3',4'}$ = 6.3 Hz), 5.35–5.41 (1H, 2t, $J_{2',3'}$ = 6.3 Hz), 6.07 (1H, 2d, $J_{1',2'}$ = 2.0 Hz), 6.51 (2H, bs), 8.03 (1H, s); ^{13}C NMR ($CDCl_3$) δ 16.2, 16.3, 16.8, 16.9, 16.9, 22.7, 22.7, 22.7, 22.8, 24.6, 24.3, 24.3, 24.4, 24.4, 25.4, 25.4, 27.0, 27.8, 42.4, 42.5, 42.6, 42.7, 44.9, 45.0, 58.8, 58.9, 59.1, 59.1, 59.2, 62.7, 63.0, 63.2, 77.2, 82.1, 82.2, 86.2, 86.3, 86.4, 87.0, 87.1, 101.6, 101.7, 113.3, 113.4, 147.5, 147.5, 147.7, 148.7, 149.6, 153.3; ^{31}P NMR ($CDCl_3$) δ 146.58, 146.87; ESI-mass *m/z* calcd for $C_{26}H_{44}N_6O_8P$ 599.2958, observed [M + H] 599.2986.

Diethyl *N*-Acetylphosphoramidate (11). Acetamide (236 mg, 4 mmol) was coevaporated four times with dry acetonitrile and finally dissolved in dry acetonitrile (40 mL). To the solution were added diethyl *N,N*-diisopropylphosphoramidite (**10**) (1.40 mL, 6 mmol) and 1*H*-tetrazole (841 mg, 12 mmol), and the mixture was stirred at room temperature for 30 min.

A 6 M solution of *tert*-butyl hydroperoxide in decane (3.3 mL, 20 mmol) was added. After the mixture was stirred at room temperature for 30 min, a 6 M solution of *tert*-butyl hydroperoxide in decane (3.3 mL, 20 mmol) was again added. After being stirred at room temperature for an additional 10 min, the mixture was diluted by addition with $CHCl_3$. The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH (100:0–99:1, v/v) to give **11** (304 mg, 40%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.37 (6H, 2t, J = 6.9 Hz), 2.13 (3H, 2s), 4.10–4.30 (4H, m, $J_{P,H}$ = 10.2 Hz), 8.99 (1H, bs); ^{13}C NMR ($CDCl_3$) δ 15.9, 16.0, 23.9, 24.0, 63.8, 63.9, 172.1, 172.1; ^{31}P NMR ($CDCl_3$) δ −1.69; ESI-mass *m/z* calcd for $C_6H_{15}NO_4P$ 196.0739, observed [M + H] 196.0731.

Diethyl *N*-(*N*-Trityl-L-prolyl)phosphoramidate (12). *N*-Trityl-L-prolinamide (712 mg, 2 mmol) was coevaporated four times with dry acetonitrile and finally dissolved in dry acetonitrile (30 mL). To the solution were added **19** (931.7 μL, 4 mmol) and 1*H*-tetrazole (420.4 mg, 6 mmol). After the mixture was stirred at room temperature for 4 h, a 6 M solution of *tert*-butyl hydroperoxide in decane (1.67 mL, 10 mmol) was added. After stirring was continued at room temperature for 10 min, the mixture was diluted by addition of $CHCl_3$. The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc–pyridine (80:20:1–65:35:1, v/v/v) to give **12** (698 mg, 71%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.82–0.86 (1H, m), 1.05–1.48 (5H, m, J = 6.9 Hz), 1.63–1.69 (1H, m), 2.97–3.06 (1H, m), 3.27–3.36 (1H, m), 3.94 (1H, m), 4.10–4.38 (2H, m, $J_{P,H}$ = 9.9 Hz), 7.14–7.27 (9H, m), 7.46 (6H, d, J = 7.6 Hz), 8.74 (1H, d, $J_{P,H}$ = 13.8 Hz); ^{13}C NMR ($CDCl_3$) δ 16.3, 16.4, 16.5, 24.5, 31.9, 50.8, 64.1, 64.2, 64.2, 64.3, 65.8, 65.9, 78.4, 126.7, 127.9, 129.2, 144.1, 177.4, 177.4; ^{31}P NMR ($CDCl_3$) δ −2.21; ESI-mass *m/z* calcd for $C_{28}H_{34}N_2O_4P$ 493.2256, observed [M + H] 493.2577.

Diethyl *N*-L-Prolylphosphoramidate Trifluoroacetic Acid Salt (13). Compound **12** (246.3 mg, 0.5 mmol) was dissolved in a 1% solution of trifluoroacetic acid in CH_2Cl_2 (5 mL). After being stirred at room temperature for 30 min, the mixture was partitioned between water and $CHCl_3$. The aqueous layer was further washed three times with $CHCl_3$ and evaporated under reduced pressure. The residue was coevaporated three times with distilled water and subjected to a column of reverse-phase C_{18}. Elution was performed with solvent system II. The fractions containing **13** were again purified by reverse-phase C_{18} column chromatography using water–acetonitrile (90:10, v/v). Lyophilization of the fractions containing pure **13** from water gave **13** (169 mg, 93%) as a white foam: ^{1}H NMR (270 MHz, D_2O) δ 1.41 (6H, t, J = 6.9 Hz), 2.17 (3H, m), 2.59–2.62 (1H, m), 3.50–3.53 (2H, m), 4.25–4.35 (4H, m), 4.55–4.58 (1H, m); ^{13}C NMR (D_2O) δ 17.98, 18.06, 26.22, 32.04, 49.14, 63.10, 63.29, 68.26, 68.33, 112.40, 116.68, 129.97, 125.26, 164.41, 164.93, 165.46, 165.98, 174.01, 174.03, 174.05; ^{31}P NMR (D_2O) δ −1.41; ESI-mass *m/z* calcd for $C_9H_{20}N_2O_4P$ 251.1161, observed [M + H] 251.0979.

***N*^{6}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N*-Acetylphosphoramidate] (14).** A mixture of acetamide (140.0 mg, 2.37 mmol) and **9** (946.3 mg, 1.58 mmol) was rendered anhydrous by coevaporation four times with dry acetonitrile and finally dissolved in dry acetonitrile (20 mL). To the mixture was added 1*H*-tetrazole (332.1 mg, 4.74 mmol), and the solution was stirred under an argon atmosphere at room temperature for 1 h. After a 6 M solution of *tert*-butyl hydroperoxide in decane (1.32 mL, 7.90 mmol) was added, stirring was continued at room temperature for an additional 10 min. The mixture was partitioned between $CHCl_3$ and 5% $NaHCO_3$. The $CHCl_3$ layer was washed twice with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH–pyridine (98:2:1, v/v/v) to give a diastereomeric mixture of **14** (386.7 mg, 43%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.18–1.28 (6H, m, J = 6.9 Hz), 1.51 (3H, 2s), 1.57 (9H, 2s), 2.04 (3H, 2s), 4.07–4.36 (5H, m), 4.95–4.99 (1H, m, 3′-H), 5.26–5.32 (1H, m, $J_{2',3'}$ = 4.9 Hz), 6.14 (1H, 2d, $J_{1',2'}$ = 2.0 Hz), 8.06 (1H, 2s); ^{13}C NMR ($CDCl_3$) δ 16.0, 16.1, 25.3, 25.4, 27.0, 27.1, 27.9, 64.0, 64.1, 64.3, 64.4, 66.9, 67.0, 67.2, 67.2, 81.5, 82.8, 86.4, 86.6, 86.6, 86.9, 87.0, 101.9, 113.8, 114.0, 147.3, 148.0, 148.1, 148.9, 149.1, 149.3, 149.7, 153.2, 153.4, 178.5; ^{31}P NMR ($CDCl_3$) δ −1.37; ESI-mass *m/z* calcd for $C_{22}H_{34}N_6O_{10}P$ 573.2074, observed [M + H] 573.2018.

8-Oxoadenosine 5′-(Ethyl *N*-Acetylphosphoramidate) (15). Compound **14** (224 mg, 0.39 mmol) was dissolved in 80% formic acid (3.9 mL). After being stirred at room temperature for 12 h, the mixture was diluted by addition of distilled water. The aqueous solution was washed three times with EtOAc, evaporated under reduced pressure, and coevaporated three times with distilled water. The residue was dissolved in a small amount of distilled water and subjected to a column of C_{18} using medium-pressure reverse-phase silica gel column chromatography. Elution with solvent system III followed by rechromatography eluted with water–acetonitrile (90:10, v/v) gave a diastereomeric mixture of **15** as a white foam (53.5 mg, 32%): ^{1}H NMR (270 MHz, D_2O) δ 1.13–1.22 (3H, m, J = 6.9 Hz), 1.98 (3H, s), 3.95–4.09 (2H, m, $J_{P,H}$ = 14.2 Hz), 4.16–4.17 (1H, m), 4.28–4.37 (2H, m), 4.58–4.65 (1H, m), 5.07–5.10 (1H, m), 5.78 (1H, d, $J_{1',2'}$ = 4.0 Hz), 7.96 (1H, s); ^{13}C NMR (D_2O) δ 17.8, 17.9, 25.7, 25.8, 67.9, 67.9, 69.0, 69.1, 69.2, 72.1, 73.3, 73.4, 83.8, 83.9, 83.9, 84.0, 88.9, 89.0, 106.3, 148.8, 149.4, 153.4, 154.9, 178.4, 178.5, 178.6; ^{31}P NMR (D_2O) δ −0.30, −0.44; ESI-mass *m/z* calcd for $C_{14}H_{22}N_6O_8P$ 433.1237, observed [M + H] 433.1247.

***N*^{6}-*tert*-Butoxycarbony-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N*-(*N*-Trityl-L-prolyl)phosphoramidothioate] (16).** A mixture of **4** (829 mg, 1.96 mmol) and **7b** (2.08 g, 3.91 mmol) was coevaporated four times with dry acetonitrile and finally dissolved in dry acetonitrile (30 mL). To the mixture was added MMT (568 mg, 4.89 mmol), and the solution was stirred at room temperature for 1 h. *N,N,N′,N′*-Tetraethylthiuram disulfide (1.74 g, 5.87 mmol) was added, and the mixture was stirred at room temperature for 3 h. The solution was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc–pyridine (70:30:1, v/v/v) to give a diastereomeric mixture of **16** (1.03 g, 59%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.62–1.38 (10H, m), 1.47–1.50 (3H, 2s) 1.54–1.57 (9H, 2s), 2.86–2.95 (1H, m), 3.18–3.28 (1H, m), 3.82–3.90 (2H, m), 4.07–4.45 (4H, m), 5.05 (1H, m), 5.36–5.42 (1H, m), 6.17–6.22 (1H, d) 6.48 (2H, bs,) 7.09–7.21 (9H, m), 7.36–7.44 (6H, m), 8.09–8.11 (1H, 2s); ^{13}C NMR ($CDCl_3$) δ 15.7, 15.8, 15.8, 15.9, 24.1, 24.2, 25.3, 25.3, 26.9, 26.9, 27.8, 31.2, 31.3, 50.4, 50.5, 64.1, 64.2, 64.3, 64.4, 65.4, 65.4, 77.3, 81.9, 81.9, 82.8, 82.9, 86.4, 86.4, 87.0, 87.1, 101.7, 101.8, 113.5, 126.3, 127.6, 127.7, 128.9, 143.7, 143.8 147.3, 147.3, 147.9, 148.6, 148.7, 149.5, 153.3, 175.7, 175.8; ^{31}P NMR ($CDCl_3$) δ 63.15, 63.22; ESI-mass *m/z* calcd for $C_{44}H_{53}N_7O_9PS$ 886.3363, observed [M + H] 886.3339.

8-Oxoadenosine 5′-(Ethyl *N*-L-Prolylphosphoramidothioate) (17). Compound **16** (1.03 g, 1.16 mmol) was dissolved in 80% formic acid (10 mL), and the mixture was stirred at room temperature for 12 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated three times with distilled water to remove the last traces of formic acid. The residue was chromatographed on a column of C_{18} with water–acetonitrile (100:0–96:4) followed by lyophilization from its aqueous solution to give a diastereomeric mixture of **17** (209 mg, 36%) as a white foam: ^{1}H NMR (270 MHz, D_2O) δ 0.96–1.03 (3H,

t) 1.84−1.96 (3H, m), 2.19 (1H, m,), 3.18−3.27 (2H, m), 3.68−3.85 (3H, m), 3.94−4.08 (3H, m,), 4.51−4.59 (1H, m), 5.01−5.06 (1H, m), 5.70−5.72 (1H, d, $J_{1',2'}$ = 3.6 Hz), 7.91 (1H, s); ^{13}C NMR (D_2O) δ 17.7, 17.8, 26.4, 32.2, 32.3, 48.9, 64.8, 65.1, 65.6, 65.7, 65.8, 67.9, 68.0, 72.4, 72.5, 73.2, 84.3, 84.4, 84.5, 88.9, 89.0, 106.9, 149.0, 149.7, 153.5, 155.3, 177.7, 177.8; ^{31}P NMR (D_2O) δ 70.00, 70.05; ESI-mass *m/z* calcd for $C_{17}H_{27}N_7O_7$-PS 504.1430, observed [M + H] 504.1450.

Ethyl *N,N*-Diisopropyl-*N*-(*N*-trityl-D-prolyl)phosphorodiamidite (18). A mixture of *N*-trityl-D-prolinamide[21] (1.78 g, 5.0 mmol) and *N,N*-diisopropylammonium 1*H*-tetrazolide (514 mg, 3.0 mmol) was rendered anhydrous by coevaporation three times each with dry pyridine and dry toluene and finally dissolved in dry CH_2Cl_2 (50 mL). To the mixture was added **6b** (2.09 mL, 3.0 mmol), and the mixture was stirred under argon atmosphere at room temperature for 3 h. The solution was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes−EtOAc−pyridine (100:0:1−85:15:1, v/v/v) to give a diastereomeric mixture of **18** (2.01 g, 76%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.78−1.38 (18H, m), 1.65−1.79 (1H, m), 2.94−3.10 (1H, m), 3.21−3.32 (1H, m), 3.63−3.89 (5H, m), 7.12−7.27 (9H, m), 7.50−7.53 (6H, m), 8.09 (1H, 2bs); ^{13}C NMR ($CDCl_3$) δ 17.1, 17.2, 17.2, 17.3, 24.1, 24.3, 24.35, 24.4, 24.45, 24.5, 24.55, 24.6, 31.0, 31.1, 44.1, 44.3, 44.6, 44.5, 50.4, 50.5, 60.6, 61.0, 65.6, 65.7, 78.2, 126.2, 127.6, 129.0, 129.1, 144.4, 144.5, 178.2, 178.3, 178.4, 178.6; ^{31}P NMR ($CDCl_3$) δ 113.00, 114.51; ESI-mass *m/z* calcd for $C_{32}H_{43}N_3O_2P$ 532.3093, observed [M + H] 532.3029.

***N*^{2}-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N*-(*N*-Trityl-D-prolyl)phosphoramidate] (19).** A mixture of **4** (690 mg, 1.63 mmol) and **18** (1.73 g, 3.26 mmol) was rendered anhydrous by coevaporation four times with dry acetonitrile and finally dissolved in dry acetonitrile (30 mL). To the mixture was added MMT (473 mg, 4.08 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 1 h. A 6 M solution of *tert*-butyl hydroperoxide in decane (2.7 mL, 16.3 mmol) was added. After being stirred at room temperature for 10 min, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$−MeOH−pyridine (100:0:1−98.5:1.5:1, v/v/v) to give a diastereomeric mixture of **19** (1.17 g, 83%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.80−0.91 (1H, m), 0.94−1.61 (21H, m), 2.90−3.07 (1H, m), 3.26−3.36 (1H, m), 3.91−3.94 (1H, m), 4.20−4.50 (5H, m, $J_{P,H}$ = 10.2 Hz), 5.05−5.13 (1H, m), 5.40−5.46 (1H, m, $J_{2',3'}$ = 6.3 Hz), 6.25−6.30 (1H, 2d, $J_{1',2'}$ = 1.6 Hz), 6.61 (2H, bs), 7.13−7.32 (9H, m), 7.46 (6H, d, J = 7.9 Hz), 8.13 (1H, 2s), 8.89 (1H, 2d, J_{PNH} = 13.5 Hz); ^{13}C NMR ($CDCl_3$) δ 15.95, 16.00, 16.05, 16.1, 24.1, 25.3, 27.0, 27.7, 31.0, 31.4, 34.0, 50.4, 50.5, 64.0, 64.1, 64.15, 64.8, 65.2, 65.3, 65.35, 65.4, 67.1, 67.15, 67.2, 77.2, 77.9, 78.0, 78.1, 81.6, 81.7, 82.6, 82.8, 85.7, 85.8, 86.3, 86.9, 101.7, 101.75, 113.7, 113.7, 126.1, 126.2, 126.3, 127.5, 127.6, 128.9, 143.0, 143.9, 144.3, 147.2, 147.3, 147.8, 147.85, 148.7, 149.4, 149.5, 153.3, 177.25, 177.27, 177.31, 178.9; ^{31}P NMR ($CDCl_3$) δ −1.64, −1.98; ESI-mass *m/z* calcd for $C_{44}H_{53}N_7O_{10}P$ 870.3592, observed [M + H] 870.4179.

8-Oxoadenosine 5′-(Ethyl *N*-D-Prolylphosphoramidate) Trifluroroacetic Acid Salt (20). Compound **19** (1.12 g, 1.3 mmol) was dissolved in 80% formic acid (13 mL). The mixture was stirred at room temperature for 12 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated three times with distilled water to remove the last traces of formic acid. The residue was chromatographed on a column of C_{18} with solvent system II using medium-pressure chromatography. The fractions containing **20** were evaporated under reduced pressure. The residue was further purified by reverse-phase C_{18} chromatography with water−acetonitrile (95:5, v/v) to give a diastereomeric mixture of **20** (78 mg, 10%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.16 (3H, t, J = 6.9 Hz), 1.96 (3H, m), 2.36−2.38 (1H, m), 3.32−3.34 (2H, m), 4.04−4.14 (3H, m), 4.23−4.38 (3H, m), 4.54−4.57 (1H, m), 4.92−4.95 (1H, m, $J_{2',3'}$ = 6.3 Hz), 5.84 (1H, d, $J_{1',2'}$ = 3.0 Hz), 8.29 (1H, s); ^{13}C NMR ($CDCl_3$) δ 17.8, 17.9, 26.1, 31.8, 31.9, 49.1, 63.0, 63.2, 68.3, 68.4, 69.6, 69.7, 72.15, 72.2, 73.7, 73.8, 84.3, 84.4, 89.2, 89.3, 106.85, 106.9, 112.3, 116.6, 120.8, 125.1, 144.34, 146.7, 148.9, 154.65, 154.7, 164.1, 164.6, 165.2, 165.7, 173.8, 173.84, 173.86, 173.88; ^{31}P NMR ($CDCl_3$) δ −1.52, 1.62; ESI-mass *m/z* calcd for $C_{17}H_{27}N_7O_8P$ 488.1659, observed [M + H] 488.1680.

Ethyl *N,N*-Diisopropyl-*N*-(*N*-trityl-L-isoleucyl)phosphorodiamidite (21). A mixture of *N*-trityl-L-isoleucinamide (1.96 g, 5.0 mmol) and *N,N*-diisopropylammonium 1*H*-tetrazolide (514 mg, 3.0 mmol) was rendered anhydrous by coevaporation three times each with dry pyridine and dry toluene and finally dissolved in dry CH_2Cl_2 (50 mL). To the mixture was added **6b** (2.09 mL, 3.0 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 4 h. The solution was diluted with $CHCl_3$ and washed three times with 5% $NaHCO_3$. The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes−EtOAc−pyridine (100:0:1−95.5:0.5:1, v/v/v) to give a diastereomeric mixture of **21** (2.21 g, 75%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.63 (3H, 2t, $J_{5'',4''}$ = 7.3 Hz), 0.83 (3H, 2d, J = 6.9 Hz), 0.97−1.39 (17H, m), 2.40−2.61 (1H, m), 3.09−3.24 (1H, m), 3.45−3.63 (2H, m, J = 6.9 Hz), 3.66−3.82 (2H, m, J = 6.9 Hz, $J_{P,H}$ = 10.2 Hz), 7.13−7.32 (9H, m), 7.39−7.47 (6H, m); ^{13}C NMR ($CDCl_3$) δ 12.2, 12.3, 14.1, 14.3, 17.1, 17.2, 17.3, 24.2, 24.28, 24.30, 24.35, 24.4, 24.45, 24.5, 27.3, 27.4, 40.7, 41.0, 44.0, 44.1, 44.2, 44.3, 60,5, 60.6, 60.9, 60.95, 61.1, 61.45, 61.50, 71.9, 72.2, 77.2, 126.3, 126.5, 127.63, 127.7, 128.60, 128.61, 145.6, 146.0, 175.2, 175.4, 175.7, 175.9; ^{31}P NMR ($CDCl_3$) δ 113.53, 114.65; ESI-mass *m/z* calcd for $C_{33}H_{47}N_3O_2P$ 548.3406, observed [M + H] 548.3380.

8-Oxoadenosine 5′-(Ethyl *N*-L-Isoleucylphosphoramidate) Trifluoroacetic Acid Salt (24a). A mixture of **4** (788 mg, 1.86 mmol) and **21** (2.21 g, 3.72 mmol) was rendered anhydrous by coevaporation four times with dry acetonitrile and finally dissolved in dry acetonitrile (28 mL). To the mixture was added MMT (540 mg, 4.65 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 1 h. A 6 M solution of *tert*-butyl hydroperoxide in decane (3.10 mL, 18.6 mmol) was added. After being stirred at room temperature for 10 min, the mixture was diluted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with $CHCl_3$−MeOH−pyridine (100:0:1−98.5:1.5:1, v/v/v) to give a diastereomeric mixture of **22a** (1.17 g, 83%) as a white foam. This compound was dissolved in 80% formic acid (19 mL). The mixture was stirred at room temperature for 12 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated three times with distilled water to remove the last traces of formic acid. The residue was chromatographed on a column of C_{18} with solvent system II using medium-pressure chromatography. The fractions containing **24a** were evaporated under reduced pressure. The residue was further purified by reverse-phase C_{18} chromatography with water−acetonitrile (95:5, v/v) to give a diastereomeric mixture of **24a** (44.7 mg, 4%) as a white foam: ^{1}H NMR (270 MHz, D_2O) δ 0.89 (3H, m), 1.01−1.02 (3H, m), 1.25−1.30 (4H, m), 1.39−1.42 (1H, m), 1.99−2.06 (1H, m), 3.96−3.99 (1H, m), 4.12−4.39 (5H, m), 4.63−4.67 (1H, m), 5.04−5.08 (1H, m), 5.92−5.96 (1H, m, $J_{1',2'}$ = 2.0

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Hz), 8.37 (1H, 2s); ^{13}C NMR (D_2O) δ 13.4, 17.0, 17.85, 17.87, 17.9, 18.0, 26.2, 38.7, 61.2, 61.4, 68.4, 68.5, 69.65, 69.7, 69.8, 72.15, 72.2, 73.65, 73.7, 84.3, 84.4, 84.5, 89.2, 107.0, 107.05, 112.4, 116.7, 121.0, 125.3, 144.6, 146.85, 146.90, 149.0, 154.8, 154.9, 164.5, 165.0, 165.5, 166.1, 174.1, 174.2; ^{31}P NMR (D_2O) δ −1.32, −1.46; ESI-mass *m/z* calcd for $C_{18}H_{31}N_7O_8P$ 504.1972, observed [M + H] 504.1846.

***N*-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N*-(*N*-Trityl-D-isoleucyl)phosphoramidate] (22b).** A mixture of *N*-trityl-D-isoleucinamide **25b** (115 mg, 0.31 mmol) and **9** (277.8 mg, 0.46 mmol) was rendered anhydrous by coevaporation four times with dry acetonitrile and finally dissolved in dry acetonitrile (2.8 mL). To the mixture was added DNPT (73 mg, 0.31 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 15 min. A 1 M solution of iodine in pyridine−water (9:1, v/v, 3.1 mL) was added, and the mixture was stirred at room temperature for 30 min. The solution was diluted with $CHCl_3$, washed twice with 5% Na_2SO_3 and three times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes−EtOAc−pyridine (60:40:1−50:50:1, v/v/v) to give a diastereomeric mixture of **22b** (67.4 mg, 16%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.53−0.58 (3H, m), 0.70−0.87 (6H, m), 1.03−1.28 (5H, m), 1.48 (3H, 2s), 1.54 (9H, 2s), 2.48 (1H, m), 3.21 (1H, m), 3.91−4.30 (5H, m), 4.91−4.98 (1H, m), 5.31−5.35 (1H, m, $J_{2',3'}$ = 6.3 Hz), 6.13−6.18 (1H, m), 7.11−7.28 (15H, m), 8.07 (1H, s); ^{13}C NMR (CD_3Cl) δ 11.0, 12.1, 14.1, 14.2, 14.4, 14.5, 16.10, 16.15, 16.2, 16.25, 22.7, 23.0, 23.8, 25.5, 25.55, 27.2, 27.25, 28.0, 28.9, 29.15, 29.2, 29.3, 29.35, 29.4, 29.5, 29.7, 29.8, 30.4, 31.9, 35.9, 38.7, 41.2, 41.3, 61.15, 61.20, 61.25, 61.3, 64.0, 64.05, 64.2, 64.3, 66.9, 66.95, 66.96, 66.98, 67.01, 68.1, 72.15, 72.18, 77.21, 81.8, 81.9, 82.7, 82.75, 85.3, 85.4, 85.5, 85.6, 86.6, 86.7, 87.0, 87.05, 101.9, 102.0, 113.9, 114.0, 126.9, 127.0, 128.0, 128.6, 129.6, 129.8, 130.7, 132.3, 145.0, 145.05, 147.5, 147.6, 147.9, 147.95, 148.8, 149.7, 149.8, 153.5, 153.55, 167.5, 174.6, 174.65, 174.7, 174.75; ^{31}P NMR (CD_3Cl) δ −2.12, −2.23; ESI-mass *m/z* calcd for $C_{45}H_{57}N_7O_{10}P$ 886.3905, observed [M + H] 886.3882.

8-Oxoadenosine 5′-(Ethyl *N*-D-Isoleucylphosphoramidate) Trifluoroacetic Acid Salt (24b). Compound **22b** (67.4 mg, 0.076 mmol) was dissolved in a mixture of 10% trifluoroacetic acid−THF (1:1, v/v, 0.76 mL). The mixture was stirred at room temperature for 16 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated three times with distilled water to remove the last traces of TFA. The residue was dissolved in a mixture of 20% trifluoroacetic acid−THF (1:1, v/v, 0.76 mL). The mixture was stirred at room temperature for 16 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated three times with distilled water. The residue was chromatographed on a column of C_{18} with solvent system III using medium-pressure chromatography. The fractions containing **24b** were evaporated under reduced pressure. Further purification of the residue by reverse-phase C_{18} chromatography with water−acetonitrile (90:10, v/v) followed by lyophilization from the aqueous solution gave a diastereomeric mixture of **24b** (24 mg, 51%) as a white foam: ^{1}H NMR (270 MHz, D_2O) δ 0.81−0.88 (3H, m), 0.91−0.97 (3H, m, J = 7.3 Hz), 1.11−1.16 (4H, m, J = 6.9 Hz), 1.33−1.46 (1H, m), 1.85−1.97 (1H, m), 3.56−3.59 (1H, m), 3.84−3.95 (2H, m, J_{POCH} = 14.2 Hz), 4.18−4.19 (3H, m), 4.60−4.64 (1H, m), 5.11−5.17 (1H, m, $J_{2',3'}$ = 5.6 Hz), 5.86 (1H, d, $J_{1',2'}$ = 4.3 Hz), 8.09 (1H, s); ^{13}C NMR (D_2O) δ 13.55, 13.6, 17.3, 17.4, 17.8, 17.85, 17.9, 17.95, 26.35, 26.4, 39.15, 39.2, 63.05, 63.1, 65.7, 65.8, 67.75, 67.8, 67.85, 67.86, 72.15, 72.2, 73.1, 73.2, 84.2, 84.3, 88.75, 88.8, 107.0, 107.05, 112.4, 116.7, 121.0, 125.3, 144.6, 146.9, 146.9, 150.0, 153.6, 164.5, 165.0, 165.5, 166.1, 178.9; ^{31}P NMR (D_2O) δ −1.32, −1.46; ESI-mass *m/z* calcd for $C_{18}H_{31}N_7O_8P$ 504.1972, observed [M + H] 504.1937.

***N*-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N*-(*N*-Trityl-L-alanyl)phosphoramidate] (22c).** A mixture of *N*-trityl-L-alaninamide (**25c**) (94.2 mg, 0.29 mmol) and **9** (256 mg, 0.43 mmol) was rendered anhydrous by coevaporation four times with dry acetonitrile and finally dissolved in dry acetonitrile (2.8 mL). To the mixture was added DNPT (67.3 mg, 0.29 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 10 min. A 1 M solution of iodine in pyridine−water (9:1, v/v, 2.9 mL) was added, and the mixture was stirred at room temperature for 15 min. The solution was diluted with $CHCl_3$, washed twice with 5%Na_2SO_3 and three times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes−EtOAc (50:50, v/v/v) to give a diastereomeric mixture of **22c** (159 mg, 44%) as a white foam: ^{1}H NMR (270 MHz, CD_3Cl) δ 1.04 (3H, 2d, $J_{3'',2''}$ = 7.3 Hz), 1.21−1.29 (3H, m, J = 7.9 Hz), 1.32 (3H, 2s), 1.54 (3H, 2s), 1.60 (9H, s), 3.22−3.36 (1H, m), 3.91−4.38 (5H, m), 4.98−5.01 (1H, m, 3′-H,), 5.37−5.42 (1H, m, $J_{2',1'}$ = 2.0 Hz, $J_{2',3'}$ = 6.6 Hz), 5.46 (1H, bs), 6.19 (1H, 2d), 6.51 (2H, bs), 7.16−7.40 (15H, m), 8.12 (1H, s), 8.38 (1H, 2bs); ^{13}C NMR (CD_3Cl) δ 16.0, 16.05, 16.1, 16.2, 21.3, 21.4, 21.5, 25.5, 27.2, 28.0, 29.7, 53.6, 54.5, 63.9, 63.95, 64.0, 64.1, 67.0, 71.7, 71.8, 77.2, 81.7, 81.9, 82.6, 82.7, 85.4, 85.5, 86.6, 86.65, 87.0, 87.05, 101.9, 101.95, 113.9, 113.9, 123.6, 126.8, 126.8, 127.6, 127.95, 128.0, 128.45, 128.5, 128.6, 144.9, 145.0, 147.5, 147.95, 148.0, 148.75, 148.8, 149.6, 149.8, 153.5, 177.1, 178.8; ^{31}P NMR (CD_3Cl) δ −2.02, −2.07; ESI-mass *m/z* calcd for $C_{42}H_{51}N_7O_{10}P$ 844.3435, observed [M + H] 844.3406.

8-Oxoadenosine 5′-(Ethyl *N*-L-Alanylphosphoramidate) Trifluoroacetic Acid Salt (24c). Compound **22c** (100 mg, 0.012 mmol) was dissolved in a mixture of 10% trifluoroacetic acid−THF (1:1, v/v, 1.2 mL). The mixture was stirred at room temperature for 25 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated 3 times with distilled water to remove the last traces of TFA. The residue was dissolved in a mixture of 80% formic acid (1.2 mL). The mixture was stirred at room temperature for 5 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated three times with distilled water. The residue was chromatographed on a column of C_{18} with solvent system III using medium-pressure chromatograpy. The fractions containing **24c** were evaporated under reduced pressure. Further purification of the residue by reverse-phase C_{18} chromatography with water−acetonitrile (90:10, v/v) followed by lyophilization from the aqueous solution gave a diastereomeric mixture of **24c** (22 mg, 32%) as a white foam: ^{1}H NMR (270 MHz, D_2O) δ 1.03 (3H, t, J = 6.9 Hz), 1.37 (3H, d, $J_{3'',2''}$ = 6.9 Hz), 3.86−4.06 (4H, m), 4.19−4.23 (2H, m, 5′-H,), 4.44−4.50 (1H, m), 4.92−4.99 (1H, m, $J_{2',3'}$ = 5.3 Hz), 5.68−5.70 (1H, m, $J_{1',2'}$ = 2.6 Hz), 7.90 (1H, s); ^{13}C NMR (D_2O) δ 17.8, 17.9, 18.6, 52.4, 52.6, 68.25, 68.3, 68.35, 68.4, 69.5, 69.6, 69.7, 72.1, 72.2, 73.3, 73.4, 76.1, 83.8, 83.85, 83.9, 84.0, 88.9, 89.0, 106.7, 112.4, 116.7, 120.9, 125.2, 149.0, 149.65, 149.7, 153.5, 155.1, 155.2, 164.6, 165.1, 165.6, 166.1, 175.25, 175.5; ^{31}P NMR (D_2O) δ −0.77, −0.95; ESI-mass *m/z* calcd for $C_{15}H_{25}N_7O_8P$ 462.1502, observed [M + H] 462.1461.

***N*-*tert*-Butoxycarbonyl-2′,3′-*O*-isopropylidene-8-oxoadenosine 5′-[Ethyl *N*-(*N*-Trityl-L-methanol)phosphoramidate] (22d).** A mixture of *N*-trityl-L-methioninamide (**25d**) (91 mg, 0.23 mmol) and **9** (209 mg, 0.35 mmol) was rendered anhydrous by coevaporation four times with dry acetonitrile and finally dissolved in dry acetonitrile (3.5 mL). To the mixture was added DNPT (55 mg, 0.23 mmol), and the mixture was stirred under an argon atmosphere at room temperature for 10 min. A 1 M solution of iodine in pyridine−water (9:1, v/v, 2.3 mL) was added, and the mixture was stirred at room temperature for 20 min. The solution was diluted with $CHCl_3$,

washed twice with 5% Na_2SO_3 and three times with 5% $NaHCO_3$, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with hexanes–EtOAc (60:40, v/v/v) to give a diastereomeric mixture of **22d** (80 mg, 25%) as a white foam: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.16–1.27 (6H, m, J = 6.9 Hz), 1.48 (3H, 2s), 1.54 (9H, s), 1.86 (3H, 2s), 2.13–2.25 (2H, m), 2.33–2.43 (2H, m), 3.30 (1H, m), 3.91–4.33 (5H, m), 4.92–4.96 (1H, m), 5.31–5.34 (1H, m, $J_{2',3'}$ =4.9 Hz), 6.13 (1H, d, $J_{1',2'}$ = 6.3 Hz), 6.41 (2H, bs), 7.09–7.31 (15H, m), 8.06 (1H, s); ^{13}C NMR ($CDCl_3$) δ 15.5, 16.1, 16.2, 25.45, 25.5, 27.2, 28.0, 28.9, 33.6, 33.7, 38.7, 57.75, 57.8, 57.9, 64.0, 64.1, 64.2, 67.0, 67.1, 68.1, 71.8, 77.2, 81.7, 81.8, 82.7, 82.8, 85.4, 85.5, 86.6, 86.65, 86.9, 87.0, 101.9, 102.0, 113.9, 114.0, 126.8, 128.0, 128.45, 128.5, 130.7, 145.0, 145.1, 147.5, 147.9, 148.0, 148.75, 148.8, 149.7, 149.75, 153.4, 175.6, 175.65, 175.7; ^{31}P NMR ($CDCl_3$) δ −0.77, −0.95; ESI-mass *m/z* calcd for $C_{44}H_{55}N_7O_{10}PS$ 904.3469, observed [M + H] 904.3459.

8-Oxoadenosine 5′-(Ethyl *N*-L-Methionylphosphoramidate) Trifluoroacetic Acid Salt (24d). Compound **22d** (80 mg, 0.088 mmol) was dissolved in a mixture of 20% trifluoroacetic acid–THF (1:1, v/v, 0.88 mL). The mixture was stirred at room temperature for 24 h. The solution was diluted with distilled water and washed three times with EtOAc. The aqueous layer was collected, evaporated under reduced pressure, and coevaporated three times with distilled water to remove the last traces of TFA. The residue was chromatographed on a column of C_{18} with solvent system III using medium-pressure chromatography. The fractions containing **24d** was evaporated under reduced pressure. Further purification of the residue by reverse-phase C_{18} chromatography with water–acetonitrile (90:10, v/v) followed by lyophilization from the aqueous solution gave a diastereomeric mixture of **24d-fast** (7.4 mg, 13%) and **24d-slow** (10.5 mg, 18%) as a white foam. **24d-fast:** ^{1}H NMR (270 MHz, D_2O) δ 1.28 (3H, t, J = 7.3 Hz), 2.09 (3H, s), 2.13–2.34 (2H, m), 2.51–2.67 (2H, m), 4.13–4.29 (4H, m, $J_{P,H}$ = 8.6 Hz), 4.35–4.49 (2H, m), 4.63–4.67 (1H, m), 5.05–5.08 (1H, m), 5.96 (1H, d, $J_{1',2'}$ = 4.0 Hz), 8.36 (1H, s); ^{13}C NMR (D_2O) δ 16.7, 17.9, 18.0, 30.6, 32.2, 55.7, 55.9, 68.5, 68.6, 69.8, 69.9, 72.1, 73.7, 84.2, 84.4, 89.2, 107.0, 112.4, 116.7, 121.0, 125.2, 145.2, 147.6, 149.0, 154.9, 164.6, 165.1, 165.6, 166.1, 174.05, 174.1; ^{31}P NMR (D_2O) δ −1.31; ESI-mass *m/z* calcd for $C_{17}H_{29}N_7O_8PS$ 522.1536, observed [M + H] 522.1546. **24d-slow:** ^{1}H NMR (270 MHz, D_2O) δ 1.25 (3H, t, CH_3 of POEt, J = 7.3 Hz), 2.09 (3H, s, 4″-SCH_3), 2.13–2.34 (2H, m, 3″-H), 2.51–2.68 (2H, m, 4″-H), 4.10–4.28 (4H, m, 4′-H, 2″-H, CH_2 of POEt, $J_{P,H}$ = 8.2 Hz), 4.38–4.43 (2H, m, 5′-H), 4.62–4.66 (1H, m, 3′-H), 5.07–5.10 (1H, m, 2′-H), 5.94 (1H, d, 1′-H, $J_{1',2'}$ = 4.3 Hz), 8.34 (1H, s, 2-H); ^{13}C NMR (D_2O) δ 16.7, 17.8, 17.9, 30.6, 32.2, 55.7, 55.9, 68.4, 68.5, 69.8, 69.9, 72.2, 73.6, 84.3, 84.4, 89.1, 107.1, 112.5, 116.7, 121.0, 125.3, 145.4, 147.8, 149.0, 154.9, 164.6, 165.1, 165.7, 166.2, 174.05, 174.1; ^{31}P NMR (D_2O) δ −1.50; ESI-mass *m/z* calcd for $C_{17}H_{29}N_7O_8PS$ 522.1536, observed [M + H] 522.1567.

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Supporting Information Available: General methods; ^{1}H, ^{13}C, and ^{31}P NMR spectra of **1a–d**, **8b**, **11**, **13**, **15**, **17**, **20**, and **24a–d**; an experimental procedure for the assay of in vitro antitumor activity; and Figures 7–11 (the details of Tables 2 and 4–7). This material is available free of charge via the Internet at http://pubs.acs.org.

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