Chemical Synthesis and Properties of Conformationally Fixed Diuridine Monophosphates as Building Blocks of the RNA Turn Motif

Kohji Seio, Takeshi Wada, Kensaku Sakamoto,† Shigeyuki Yokoyama,† and Mitsuo Sekine*

Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midoriku, Japan, and Department of Biophysics and Biochemistry, Faculty of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan

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Two intramolecularly cyclized diuridine monophosphates having an amide and a carbamate linker have been synthesized for fixation of the U-turn structure found in various tRNAs and hammerhead ribozymes. The structural analysis of these cyclic dimers using CD, ^{1}H NMR, and ^{31}P NMR spectroscopy shows that they have rigid, unstacked conformations. Detailed computer simulations of their 3D structures based on molecular mechanics calculations suggest that a two-state equilibrium between a stretch and a turn conformation exists in favor of the former. The simulation also suggested that the diuridine monophosphate containing the carbamate-linker is better as a mimic of the U-turn structure than the amide-linked dimer. The enzymatic properties of these cyclized dimers are also described.

Introduction

The sharply bent structure called "U-turn" has been commonly found in the anticodon loop of tRNAs, and its structural properties have been studied in relation to their biological role.[1] A number of papers have reported conformational studies of anticodon first-letter ribonucleosides that are located at the bent point of the U-turn.[2] The latest discovery of a similar U-turn structure at the active site of the crystal structure of hammerhead ribozymes[3] indicates a new biological role of this motif. Since the importance of nucleotide sequences of this active site for the ribozyme activity has already been suggested by base mutation experiments,[4] the stereochemical requirement of this region, especially, the U-turn, should be clarified next. An effective way to study the conformational significance of the turn structure is to synthesize sterically fixed U-turn mimics. Although such a strategy has been successfully applied to the conformational study of the β-turn motif[5] in the field of peptide chemistry, so far there have been no applications in the field of nucleic acid chemistry.

We have previously reported[6] the chemical synthesis of Upmnm^{5}U, which corresponds to the 33−34th nucleotide dimer sequence, *i.e.*, the minimum U-turn unit, of minor tRNA^{Arg} of *E. coli*,[7a] to study conformational properties of the U-turn region. It was suggested that hydrogen bonding between the 2′-hydroxyl group of uridine (33rd) and the amino group of 5-[(methylamino)-methyl]uridine (34th) might be a possible interresidual interaction to stabilize the "rigid" C3′-endo conformation of the anticodon first letter, mnm^{5}U, which allows precise recognition of A and G in the codon third letter of mRNA.[7b] This possibility is discussed on the assumption that the 3D structural conformation of the U-turn is identical with that of tRNA^{Phe}.[8] It is also likely that such an interresidual interaction plays an additional role in stabilization of the sharp bend of the U-turn[9] as well as predicts the C3′-endo sugar pucker in the first letter. Therefore, our interest was focused on this cyclic structure via an interresidual hydrogen bonding, and we

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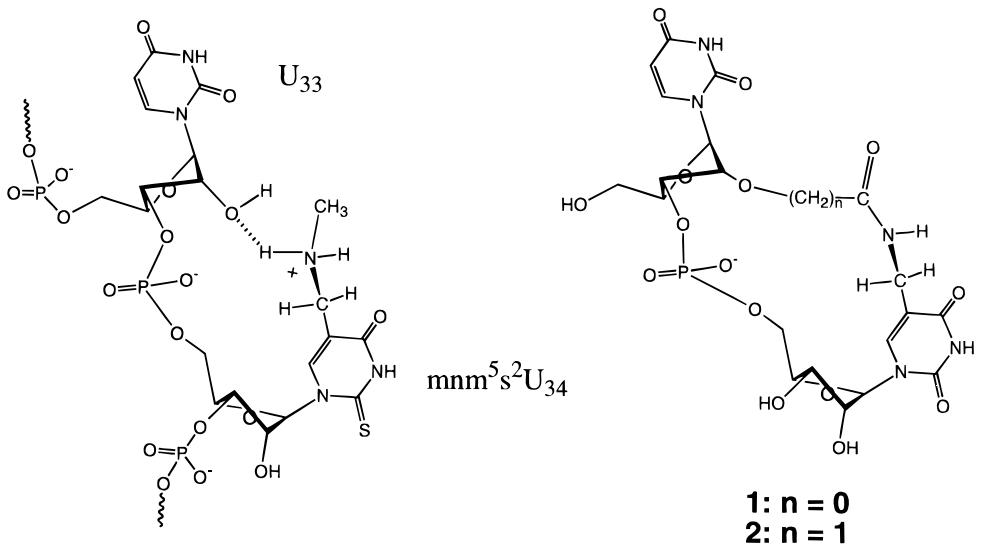
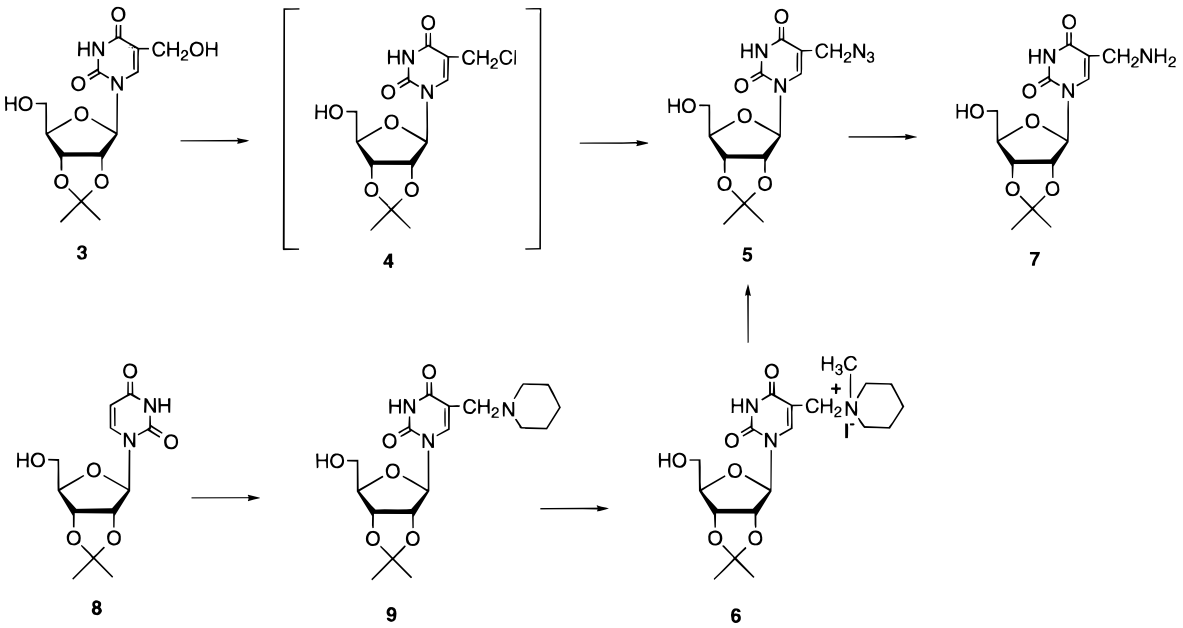


Figure 1. Left: intraresidual hydrogen bonding between the U(33) and $mnm^5s^2U(34)$ proposed by Hillen et al. Right: structures of the amide-linked and carbamate-linked cyclized dinucleoside monophosphates (**1** and **2**).

Scheme 1



designed two U-turn mimics **1** and **2** in which the hydrogen bond is replaced by an amidomethyl and a carbamate linkage, respectively, as depicted in Figure 1. A preliminary result of the synthesis of **1** has already been reported briefly by us.[10]

These two cyclic diuridylates are expected to be useful not only as starting materials for preparation of the minimum synthetic units of RNA or DNA oligonucleotides having a U-turn but also as the first mimics of the RNA turn chemically fixed by introducing a cyclic structure. In this paper, we report the detailed studies of the chemical synthesis and conformational analysis of **1** and **2**.

Results and Discussion

Synthesis of 5-(Aminomethyl)uridine Derivatives. First, we examined the synthesis of 5-(aminomethyl)-2′,3′-*O*-isopropylideneuridine (**7**), which is the common 3′-terminal nucleoside unit in the synthesis of both **1** and **2** (Scheme 1). To introduce the aminomethyl group to position 5 of 2′,3′-*O*-isopropylideneuridine, we employed 5-(chloromethyl)-2′, 3′-*O*-isopropylideneuridine (**4**)[11] as an intermediate. Compound **4** was readily obtained from 5-(hydroxymethyl)-2′,3′-*O*-isopropylideneuridine (**3**)[12] using trimethylsilyl chloride as a chlorinating agent. Compound **4** was used in situ for the next reaction without further purification because the chloromethyl group of **4** was quite labile during chromatography, producing a considerable amount of starting material **3**. Direct amination of **4** with aqueous NH_3 was unsuccessful because of further *N*-alkylation of the desired product and competitive hydrolysis of the starting material. Shiau et al. reported the successful chemical synthesis of 5-(aminomethyl)-2′-deoxyuridine by using the correspond-

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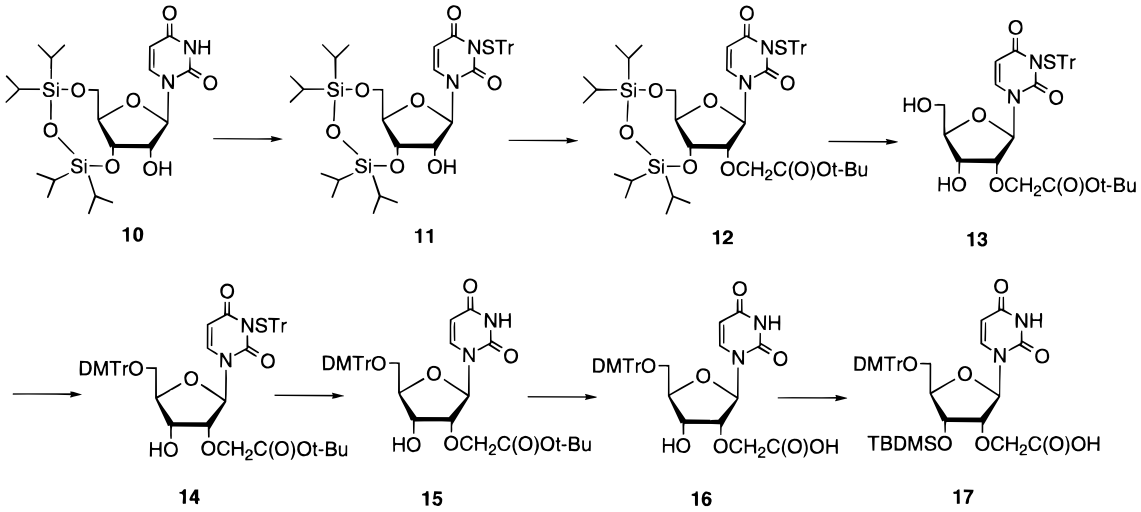
Scheme 2



ing 5-(azidomethyl)-2′-deoxyuridine derivative as an intermediate.[13] Therefore, the 5-(azidomethyl)uridine derivative **5** was prepared in an overall yield of 50% from **3** by treatment of **4** with NaN_3. Compound **5** was also synthesized in 53% yield by the azidation of the *N*-methyl quaternary salt **6** of 2′, 3′-*O*-isopropylidene-5-[(piperidin-3-yl)methyl]uridine (**9**), which was prepared by the Mannich reaction of 2′,3′-*O*-isopropylideneuridine (**8**).[14]

We have tested a number of methods to reduce the azide group into an amino group.[15] Consequently, the best result was obtained by employing Letsinger's method[15b] using 1.6 equiv of triphenylphosphine as a reducing agent. This reduction gave **7** in 71% yield.

Synthesis of Amide-Linked Diuridine Monophosphate 1. For the preparation of a 5′-terminal uridine unit of cyclic dinucleoside monophosphate **1**, we required 2′-*O*-[(alkoxycarbonyl)methyl]uridine derivatives as key intermediates. A procedure for the chemical synthesis of such 2′-*O*-[(alkoxycarbonyl)methyl]ated uridine derivatives by use of suitably protected riboses as starting materials has been reported by Haner et al.[16] Since this method involved a multistep reaction, we searched for a more straightforward procedure from a uridine derivative. Treatment of a 3′,5′-*O*-silylated uridine derivative **10** with NaH in THF followed by alkylation using ethyl bromoacetate resulted in formation of a considerable amount of N^3-alkylated product, and the desired *O*-alkylated product was obtained in 46% yield. To avoid the N^3-alkylation, we employed the triphenylmethanesulfenyl (TrS) group[17] as a uridine imide protective group. Compound **10** was converted to the *N*-protected uridine derivative **11** (Scheme 2) by phase-transfer catalysis with triphenylmethanesulfenyl chloride according to the procedure described previously.[18] Treatment of **11** with NaH followed by the successive alkylation with *tert*-butyl bromoacetate gave the 2′-*O*-[(butoxycarbonyl)methyl]-uridine derivative **12** in 78% yield. The 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl (TIPDS) group of **12** was removed by the use of TBAF in the presence of 2.2 equiv of acetic acid[19,20] to give the diol **13** in 98% yield. The usual dimethoxytritylation of **13** gave the 5′-masked product **14** in 88% yield. The TrS group of **14** was then removed to give compound **15** in 70% yield according to the procedure reported previously using 2.5 equiv of tributyltin hydride in the presence of AIBN as an initiator.[18] The *tert*-butyl ester group of **15** thus obtained was hydrolyzed in 0.2 M KOH−pyridine, and the resulting intermediate **16** was allowed to react with 2.9 equiv of *tert*-butyldimethylsilyl chloride in the presence of 2.9 equiv of imidazole to give the 5′-terminal nucleoside unit **17** in an overall 78% yield from **15**.

The 3′-*O*-silylated uridine derivative **17** was converted to the succinamido ester **18** by the reaction with *N*-hydroxysuccinimide in the presence of *N*-ethyl-*N*′-[2-(dimethylamino)propyl]carbodiimide hydrochloride. The successive aminolysis of **18** with 1.2 equiv of **7** gave compound **19** in 93% yield, as shown in Scheme 3. Phosphorylation of **19** with cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate in the presence of isodurenedisulfonyl dichloride (DDS)[21] and 1*H*-tetrazole resulted in formation of compound **20** in 82% yield. One of the two phenylthio groups was removed from **20** under weakly basic conditions (NEt_3−H_2O−pyridine), and the TBDMS group was subsequently removed by treatment with TBAF. In situ cyclization of the intermediate **21** using DDS in the presence of 1*H*-tetrazole gave the fully protected cyclic diuridine monophosphate **22** in 50% yield via a two-step reaction from **20**.

Finally, compound **22** thus obtained was deprotected by treatment with 20 equiv of bis(tributyltin) oxide[22] to

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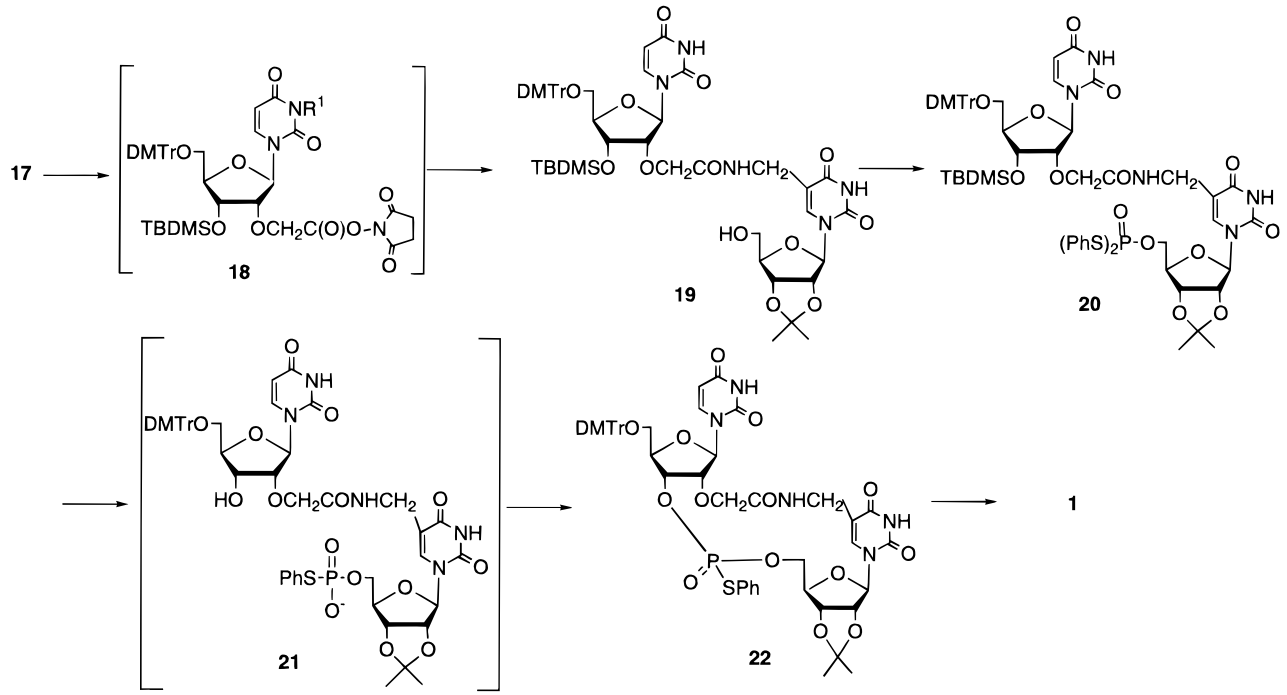
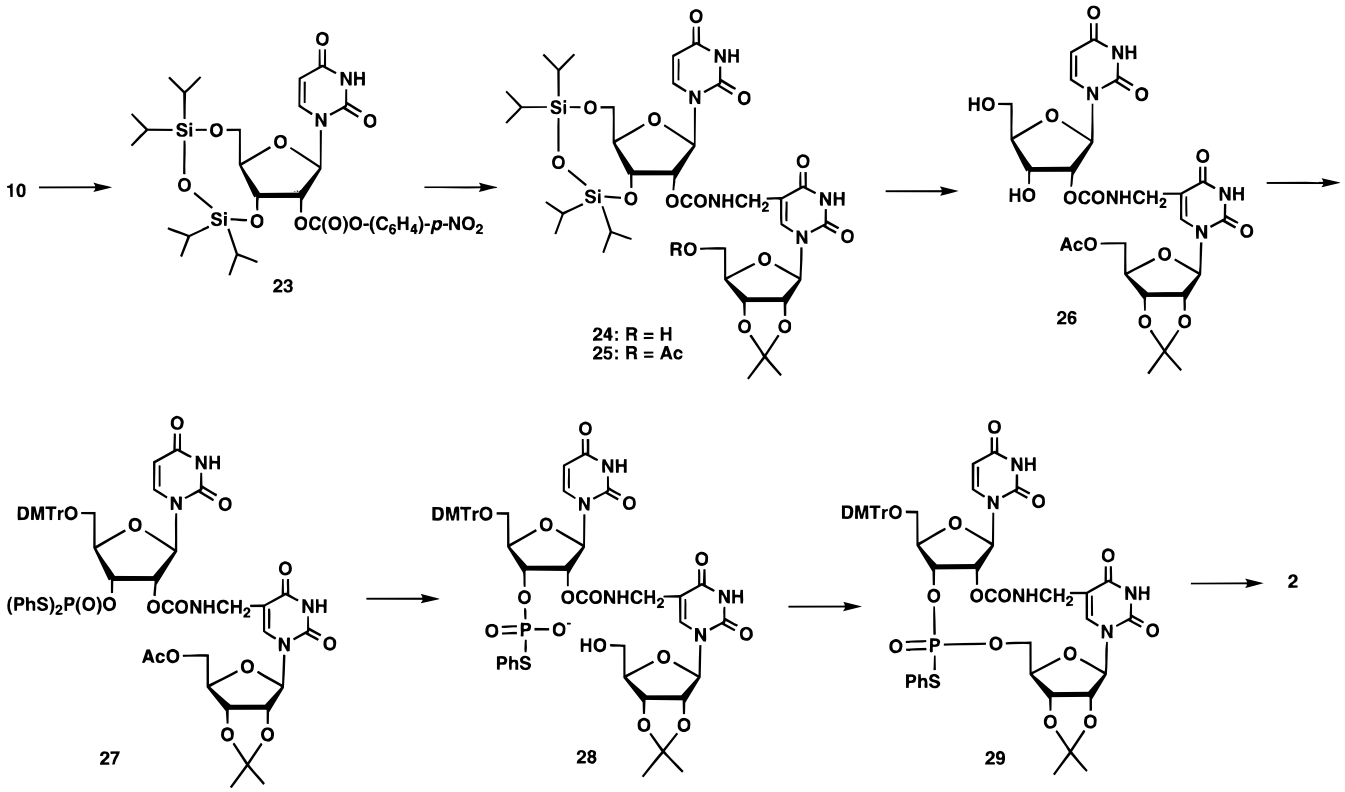
Scheme 3



Scheme 4



remove the phenylthio group followed by hydrolysis of the isopropylidene group under acidic conditions (60% formic acid at rt) to give the desired amide-linked cyclic diuridine monophosphate **1** in 61% yield. The chemical structure of **1** was confirmed by ^{1}H, ^{31}P, ^{13}C, $^{1}H-^{1}H$ COSY, and NOESY NMR spectroscopic analysis.

Synthesis of Carbamate-Linked Diuridine Monophosphate. Next, we examined the synthesis of the carbamate-linked cyclic diuridine monophosphate **2** as shown in Scheme 4. As the 5′-terminal nucleotide unit of **2** we employed a 2′-*O*-carbonate ester derivative **23**, which has a reactive *p*-nitrophenyl carbonate function. Reaction of compound **10** with 1.2 equiv of *p*-nitrophenyl chloroformate[23] in toluene in the presence of pyridine gave **23** in 96% yield.

Condensation of **23** with **7** afforded the protected dinucleoside **24** in 89% yield. The remaining 5′-hydroxyl

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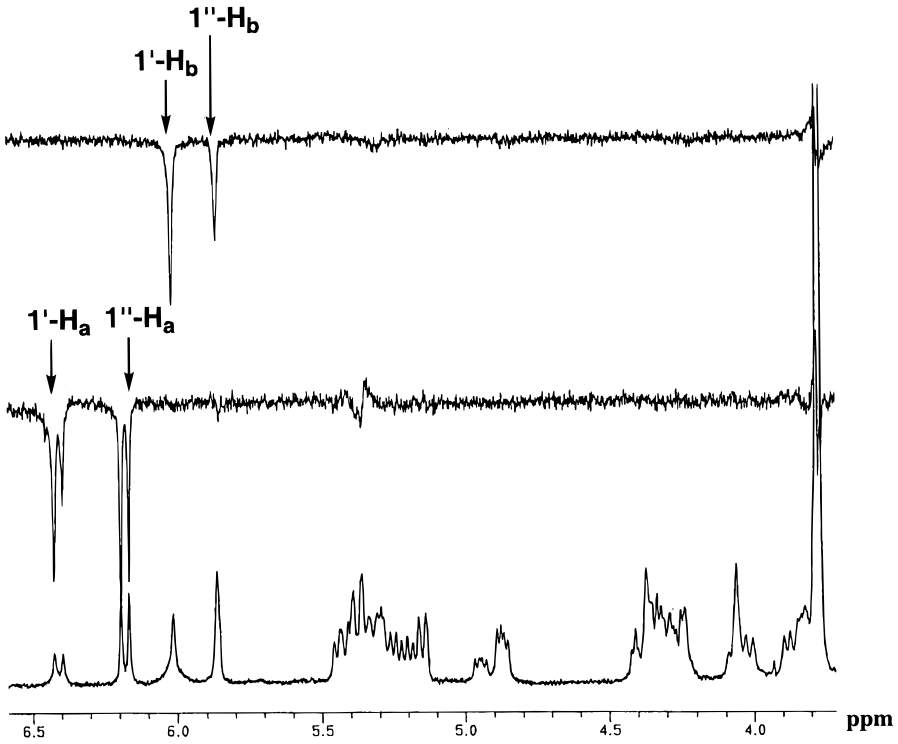

Figure 2. ^{1}H NMR saturation transfer spectra of 27 measured at 270 MHz. H_a and H_b mean that the protons are attached to the 5′- and 3′-terminal nucleotide residues, respectively. The spectra recorded on saturating the 1′-H_b signal (top) or 1′-H_a (center) are shown with the spectrum without irradiation (bottom).

group of **24** was protected by acetylation with acetic anhydride in pyridine to give the fully protected diuridine carbamate derivative **25** in a nearly quantitative yield. Then, the TIPDS group of **25** was removed by TBAF to give the diol **26** in 96% yield. Although **26** showed a single spot on TLC after purification by silica gel column chromatography, its ^{1}H NMR spectrum showed the presence of two components. It is well-known that carbonyl-containing groups, such as acetyl, benzoyl, and carbonate groups, attached to one of the two hydroxyl groups of the *cis*-diol function in ribonucleosides, tend to migrate easily to the neighboring hydroxyl group.[24] Therefore, it should be carefully checked if the carbamate group of **26** migrated from the 2′-hydroxyl group to the proximal 3′-hydroxyl group in the 5′-terminal nucleoside upon desilylation, giving rise to a mixture of the 2′- and 3′-carbamate derivatives. As discussed below, however, further analysis using ^{1}H NMR disclosed that the two isomers observed are not a pair of regioisomers but an equilibrium mixture of isomers that are probably rotamers around the O═C−N−C bond of the carbamate linkage. The 5′-hydroxyl group of **26** was further blocked with the 4,4′-dimethoxytrityl group, and the 3′-hydroxyl group was phosphorylated by using PSS-DDS-1*H*-tetrazole to give the fully protected diuridine monophosphate **27** in 88% yield via two steps from **26**. The ^{1}H NMR spectrum of **27** showed an isomeric pattern similar to that of **26** as described in the foregoing.

To ascertain the presence of the rotamers, saturation transfer ^{1}H NMR experiments of **27** were carried out.[25] These analyses as depicted in Figure 2 showed the unequivocal saturation transfers both from the 1′-H_a to the 1″-H_a signal and from the 1′-H_b to the 1″-H_b signal. These observations clearly proved that these two isomers are in slow equilibrium with each other within an NMR time scale.

One of the two phenylthio groups and the acetyl group of **27** were removed under alkaline conditions (0.2 M KOH−pyridine, 1:1, v/v), and the resulting intermediate **28** was cyclized by using DDS and 1*H*-tetrazole to give the fully protected cyclic diuridine monophosphate **29** in 49% yield. Finally, compound **29** was deprotected using the same procedure as employed in the synthesis of **1** to give the carbamate-linked diuridine monophosphate **2** in 58% yield.

The ^{1}H and ^{31}P NMR spectra of **2** also showed splitting patterns similar to those of compounds **26** and **27** as noted above. Therefore, ^{31}P and ^{1}H NMR spectra were measured at various temperatures. In the ^{31}P NMR spectra, the two signals approached closer with an increase of the temperature. Finally, they converged to a single peak at the position of 1.14 ppm at 80 °C as shown in Figure 3.

These phenomena also supported the idea that the two components in the final product are a set of equilibrium isomers. A similar conclusion was obtained from analysis of the ^{1}H NMR signals, especially of the 6-H proton signals of the 3′-terminal uridine. Although they were apart from each other (7.38 and 7.89 ppm) at 25 °C, they were melted into the baseline as the temperature was raised to 70 °C.

Properties of Cyclized Diuridine Monophosphates toward Nucleases 1 and 2. We examined the sensitivity of the cyclized diuridine monophosphates toward three enzymes of snake venom phosphodiesterase

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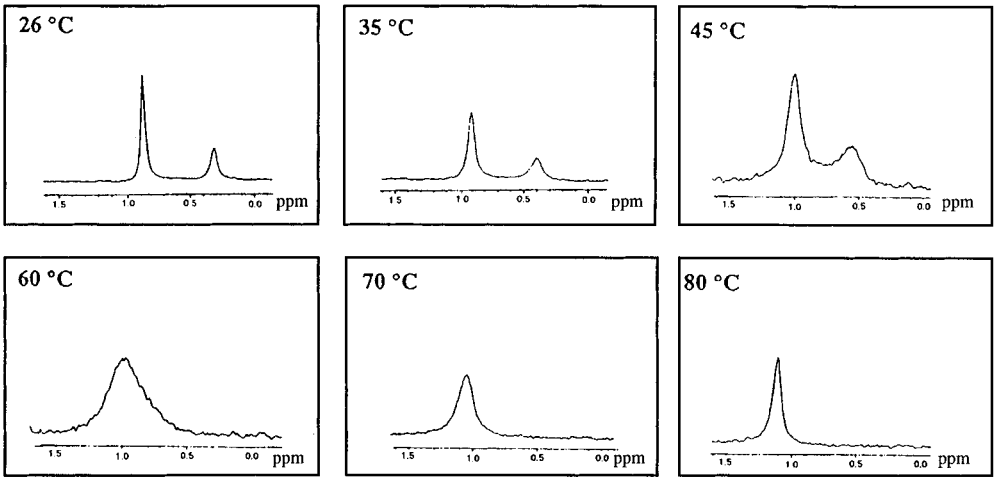

Figure 3. ^{31}P NMR spectra of **27** in $CDCl_3$ at various temperatures.

Table 1. Nucleases Sensitivity of 1 and 2[a]

	SVP	CSP	NP1
1	stable	stable	digested
2	stable	stable	stable

[a] Key: SVP, snake venom phosphodiesterase; CSP, calf spleen phosphodiesterase; NP1, nuclease P1. The reaction conditions are described in the Experimental Section.

(SVP), calf spleen phosphodiesterase (CSP), and nuclease P1 (NP1). These results are summarized in Table 1.

The amide-linked dimer **1** was not digested by SVP and CSP at all even after prolonged reaction time (4 days). In contrast, this dimer was digested by NP1 completely after 18 h to give a new single peak on HPLC that was expected to be the ring-opened product **30**. With regard to these results, some research groups have reported the interesting observation that some of the chemically synthesized and naturally occurring cyclized nucleoside diphosphates have similar tolerance and sensitivity toward enzymes as described here.[26] More interestingly, there was a marked difference in sensitivity to NP1 between **1** and **2**. As shown in Table 1, the carbamate-linked dimer **2** was resistant to SVP, CSP, and even to NP1 that could cleave the phosphodiester linkage of **1**. Since the structural difference between **1** and **2** is only one methylene group, the observed difference in their sensitivities in the NP1 digestion assay probably resulted from a subtle conformational difference between **1** and **2**. Therefore, we next studied the conformational property of these cyclic diuridine monophosphates.

Conformational Studies Using Circular Dichroism Analysis. In Figure 4 the CD spectra of **1**, **2**, and uridylyl(3′−5′)uridine (UpU) at various temperatures are shown.

The most significant were the peaks around 270 nm, which reflect the orientation of the base moiety of **1**, **2**, and UpU. UpU exhibited a striking temperature dependence of the positive Cotton effect around 270 nm changing its peak intensity, while little temperature dependence was observed in both **1** and **2**. These results could be undoubtedly explained in terms of the relative conformational rigidity of **1** and **2** compared to UpU. It should be noted that the observed conformational rigidity of **1** and **2** is suitable for our purpose to introduce stable turn structures into RNA or DNA oligomers and to control their 3D structure. There are two different aspects in the spectra of **1** and **2**. One is the positive Cotton effect around 200 nm of **2** that can be attributed to the carbamate linkage, and the other is the larger negative Cotton effect around 240 nm of **2** than that of **1**. The difference in Cotton effect around 240 nm could be due to the spatial geometry of the 5′- and 3′-terminal bases because the Cotton effect around 240 nm is commonly used as a sensitive indicator to discriminate the A- and B-type DNA duplexes.[27] Therefore, these CD spectra reflect the conformational difference between **1** and **2** which could be the origin of the different stability of these cyclized diuridine monophosphates toward NP1.

Conformational Studies Using ^{31}P NMR Analysis. Various X-ray crystallographic studies have revealed that, in the usual A-type RNA double helixes, the P−O ester bonds (ζ, α) of all ribonucleotide residues are in the energetically most favorable conformation (g^-, g^-).[28] To stabilize the RNA turn structure, the conformation of the two P−O ester bonds must be perturbed from normal (g^-, g^-) to (g^-, t), (t, g^-), (g^+, t), or (t, g^+) [These four kinds of conformers having the t and g^+ (or g^-) conformations are represented as gt/tg in this paper]. Such gt/tg conformers have been found, for example, in the crystal structure of *E. coli* $tRNA^{Phe}$ at the position of U(33)pG(34) with the dihedral angles of (g^-, t). To determine the dihedral angles around the P−O ester bond using NMR spectroscopy, however, the Karplus equation, which has been widely used in conformational studies of nucleic acids, could not be used because of the absence of the corresponding three-bond coupling constants, i.e., O−P−O−C. On the other hand, Gorenstein et al. have reported the experimental and theoretical evidence of the correlation between the dihedral angle and ^{31}P NMR chemical shift of the O−P−O−C bond and its application to the conformational analysis of oligonucleotides, DNA double strands, and DNA−protein complexes.[29] According to their work, the ^{31}P NMR chemical shifts of the inter-

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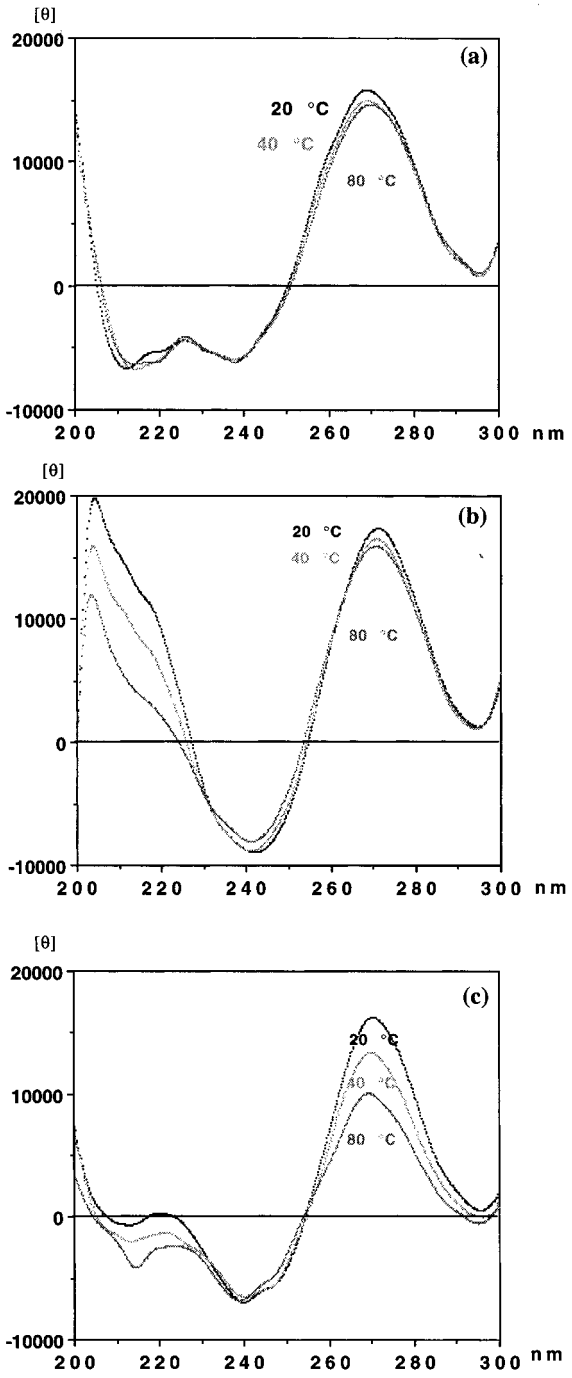

Figure 4. CD spectra of amide-linked **1** (a), carbamate-linked **2** (b) and UpU (c) in 10 mM sodium phosphate (pH 7.0).

nucleotidic phosphates are shifted upfield (∼−1 ppm) when they are in the normal (g^-, g^-) state and shifted downfield (∼+1 ppm) when the fractional population of the gt/tg conformer increases. Temperature dependence of the ^{31}P NMR chemical shifts of **1**, **2**, and 2′-*O*-methyluridylyl(3′−5′)uridine (UmpU) as a reference compound is shown in Figure 5. The chemical shifts of **2** plotted in Figure 5 were obtained by averaging those of the two rotamers described above.

The ^{31}P NMR chemical shifts of UmpU varied linearly from −0.33 to +1.07 ppm with increasing temperature (from 26 to 80 °C). This observation can be interpreted as the result of the conformational transition of the P−O bonds from the energetically stable (g^-,g^-) to the unstable gt/tg. Although a similar trend was observed

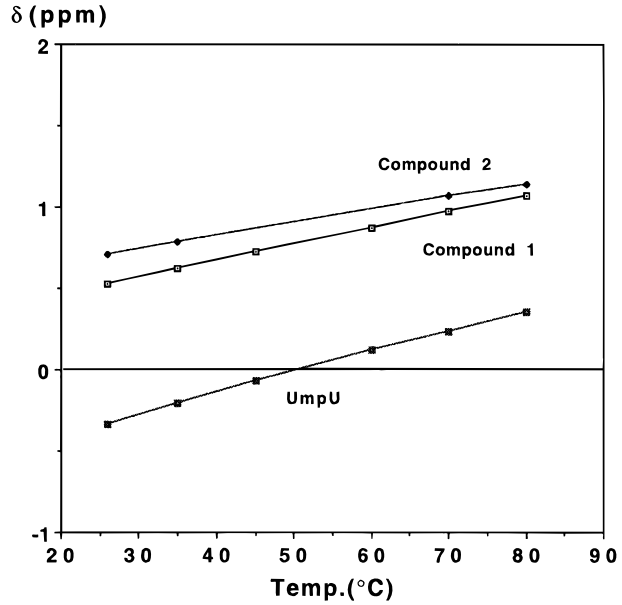

Figure 5. Temperature dependence of the ^{31}P NMR chemical shifts of **1**, **2**, and UmpU in 10 mM sodium cacodylate (pH 7.0).

Table 2. ^{1}H−^{1}H Coupling Constants of 1 Determined by 400 MHz NMR[a]

	J (Hz)	
protons	5′-terminal nucleotide residue	3′-terminal nucleotide residue
1′,2′	4.6	4.3
2′,3′	4.3	n.d.
3′,4′	2.8	n.d.
4′,5′	3.8	n.d.
4′,5″	2.8	n.d.

[a] The coupling constants that were not determined because of the peak overlap are described as n.d.

in the case of both **1** and **2**, the lines of ^{31}P chemical shifts drawn in Figure 5 show parallel shifts to lower field by 0.8 ppm for **1** and 0.9 ppm for **2** compared to that of the UmpU upon heating from 26 to 80 °C. These low-field shifts suggest that the fractional population of the gt/tg conformers around the P−O ester bonds are increased both in the amide- and carbamate-linked dimers relative to UmpU. The amplitude of the chemical sift variation $\Delta\delta = \delta(80\ °C) - \delta(26\ °C)$ was expected to reflect the conformational rigidity of these compounds. The order $\Delta\delta$ (UmpU) = 0.69 ppm > $\Delta\delta$ (amide) = 0.54 ppm > $\Delta\delta$ (carbamate) = 0.43 ppm suggested that the carbamate-linked dimer is conformationally most rigid among them, the rigidity of the amide-linked dimer ranks second, and the UmpU is the most flexible. This order is in good agreement with the structural consideration based on the number of ring members and the result of CD studies described above.

Conformation Analysis of 1 and 2 Using ^{1}H NMR and NOESY Spectra. The ^{1}H−^{1}H coupling constants of compound **1** were obtained by using 400 MHz NMR. These values are listed in Table 2.

The ribose conformation of the 5′- and 3′-terminal nucleotides was analyzed using the $J_{1',2'}$ and $J_{3',4'}$ according to the formula $P(C2'\text{-endo}) = J_{1',2'}/(J_{1',2'} + J_{3',4'})$[30] where P(C2′-endo) represents the population of the C2′-endo

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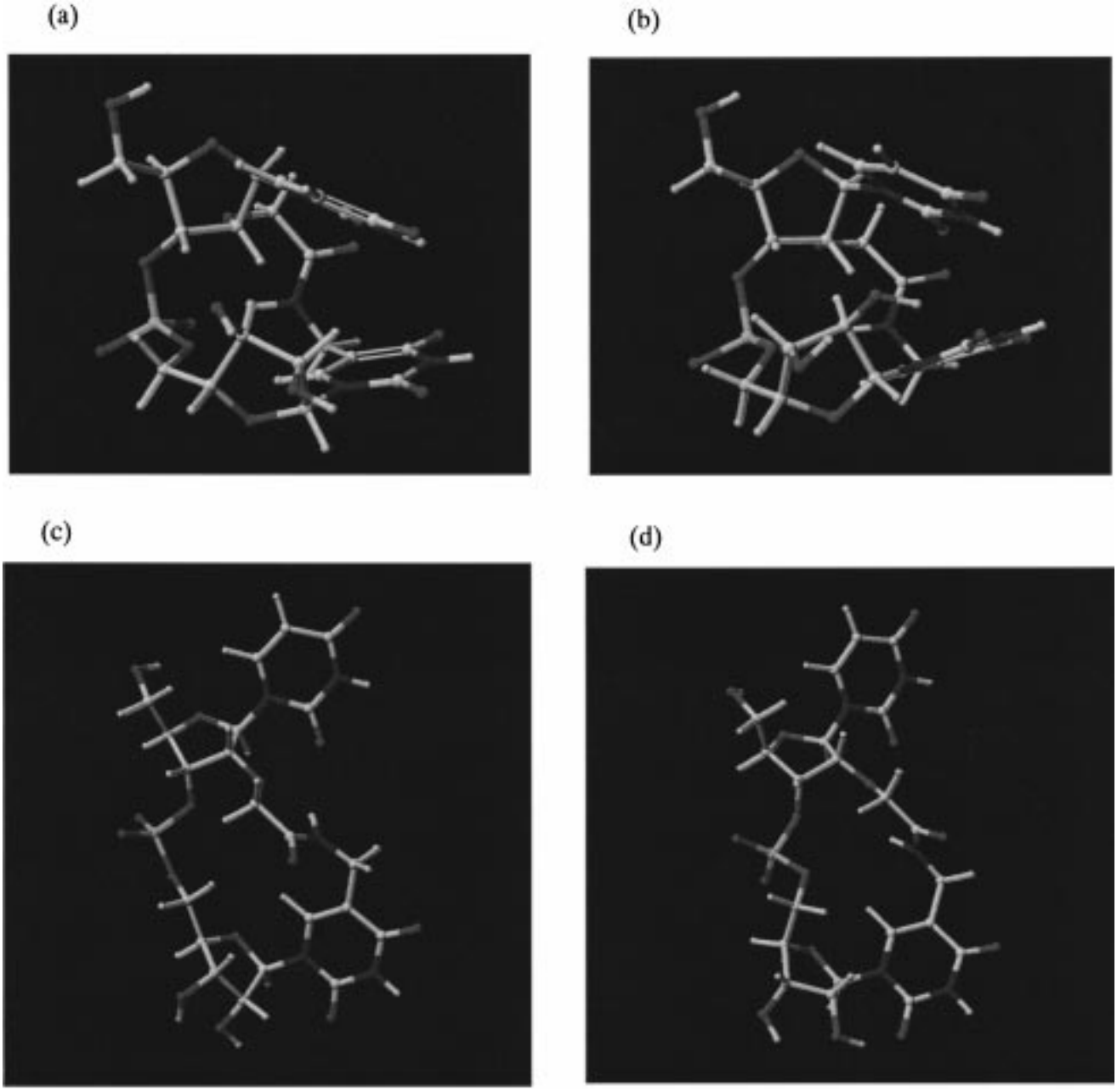

Figure 6. Low-energy structures of **1** calculated by fixing the 5′-terminal and 3′-terminal ribose conformations in C3′-endo and C3′-endo (a), C3′-endo and C2′-endo (b), C2′-endo and C3′-endo (c), and C2′-endo and C2′-endo (d), respectively.

conformation. The *P*(C2′-endo) calculated was 62% for the 5′-terminal nucleotide.

The corresponding coupling constants of compound **2** except for the $J_{1',2'}$ of both the 5′- and 3′-terminal residues of the major equilibrium isomer were not determined unambiguously because of the broadening and overlapping of the signals induced by the slow exchange. The $J_{1',2'}$ values of the 5′- and 3′-terminal residues were 8.0 and 5.1 Hz, respectively. These relatively larger values indicate that the conformation of the riboses of compound **2** is stabilized in the C2′-endo conformation.

To study the conformation of compound **1** and **2**, their NOESY spectra were measured at 500 MHz. All cross peaks observed, however, were attributed to those of interresidual interactions, and no quantitative information about the spatial geometry between the 3′-terminal and 5′-terminal nucleotide units was obtained from the NOESY spectra for either **1** or **2**. These results suggest the unstacked conformation in which the distance between the 5′- and 3′-nucleotide residues is relatively long.

3D Model Building Using Molecular Mechanics Calculations. The detailed 3D structure models of **1** and **2** were constructed by the molecular mechanics calculation using MacroModel program version 4.5.[31] The all-atom AMBER*[32] force field was used with the constant dielectric constant ϵ of 1.0 and with the implicit solvent model, GB/SA.[33] The conformation search was done by using four independent series of simulations in

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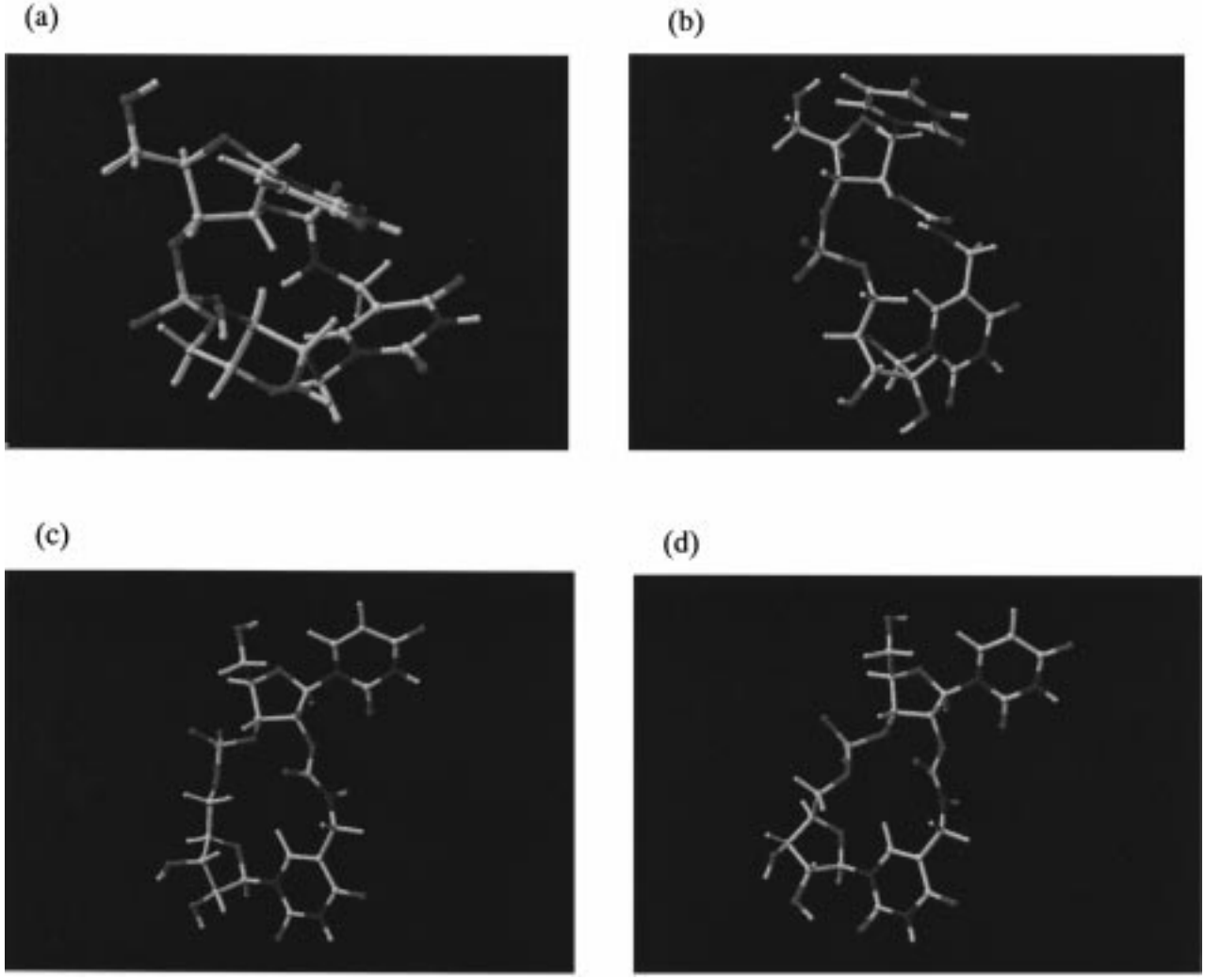

Figure 7. Low-energy structures of the trans rotamer of carbamate-linked dimer **2** calculated by fixing the 5′-terminal and 3′-terminal ribose conformations in C3′-endo and C3′-endo (a), C3′-endo and C2′-endo (b), C2′-endo and C3′-endo (c), and C2′-endo and C2′-endo (d), respectively.

which the 5′- and 3′-terminal ribose conformations were fixed to C3′-endo−C3′-endo, C3′-endo−C2′-endo, C2′-endo−C3′-endo, and C2′-endo−C2′-endo each during the simulations. In each series, 5000 conformers were generated by using the MCMM option program,[32] and the low-energy conformers (within 12 kJ/mol from the lowest energy structures) were sampled in each simulation.

Shown in Figure 6 are the lowest energy structures of amide-linked dimer **1**. Two classes of structures, which were highly correlated with conformation of the 5′-upstream ribose, were obtained. When the 5′-terminal ribose was fixed in the C3′-endo conformation, the simulations gave the "turn" conformations, as shown in Figure 6a (−882.38 kJ/mol) and 6b (−875.71 kJ/mol), in which the relative direction of the glycosyl bonds of the 5′- and 3′-terminal nucleotides was reversed and the distance between the two nucleotide residues was short. While the other two series of simulations obtained by fixing the 5′-terminal ribose conformation in the C2′-endo conformation gave the "stretch" conformations, as shown in Figure 6c (−902.83 kJ/mol) and d (−893.80 kJ/mol),[34] in which the 5′- and 3′-terminal nucleotide units were oriented almost in the same direction and the distance between them was long. The phosphate backbone conformations (ζ, α) were (g^+, g^+), (g^+, t), (t, g^+), and (g^-, t) for the structures depicted as Figure 6a−d, respectively. Those conformational properties are summarized as the dominance of the gt/tg conformations that are in agreement with those deduced from the ^{31}P NMR study described above. The NOESY and ^{1}H NMR spectra of **1** described in the preceding section indicated that **1** has a predominant conformation in which the ribose conformation was C2′-endo and the distance between the 5′- and 3′-terminal nucleotide units was long. The structures depicted in Figure 6c,d satisfy these two properties. Therefore, the solution structure of **1** should be pictured as an equilibrium mixture of the "turn" (Figure 6a,b) and "stretch" (Figure 6c,d) conformers in favor of the latter. Although the turn conformation is expected to be minor from this computer simulation, the two-state equilibrium between the turn and stretch conformations of **1** suggests the relative stability of the turn structure in this cyclized

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(34) The energy values were calculated for the structures shown in the figures after removing the constraints of the ribose. Even when the structures were energy reminimized without the constraints, the structural differences were small, rms < 0.3 Å, between the structures before and after the reminimization procedure.

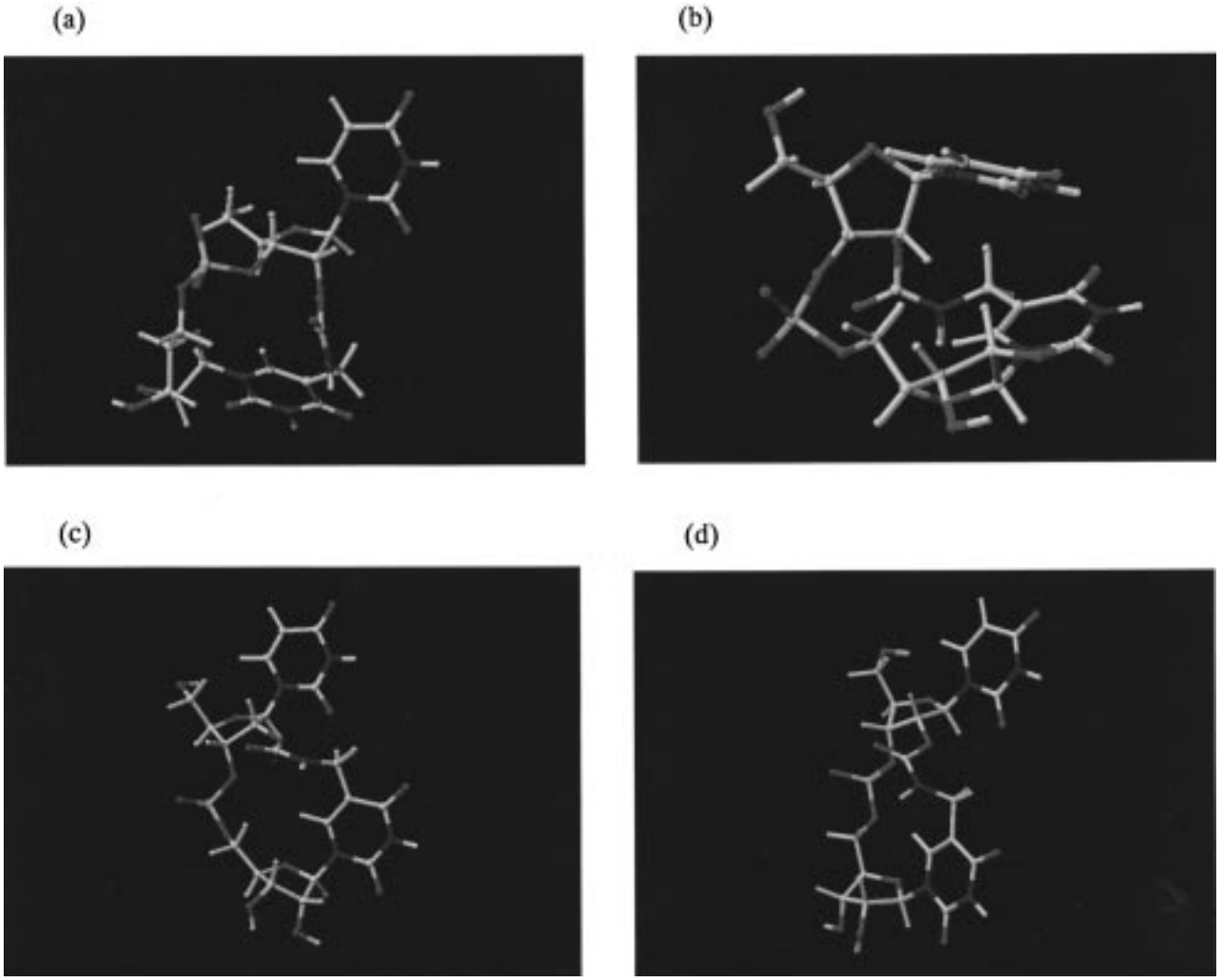

Figure 8. Low-energy structures of *cis*-carbamate-linked compound **2** calculated by fixing the 5′-terminal and 3′-terminal ribose conformations in C3′-endo and C3′-endo (a), C3′-endo and C2′-endo (b), C2′-endo and C3′-endo (c), and C2′-endo and C2′-endo (d), respectively.

dinucleoside monophosphate over that in the noncyclized dinucleoside monophosphate because many more types of conformations other than turn or stretch can be assumed in the noncyclized usual dinucleoside monophosphate.[35] The conformation properties of the turn structure (Figure 6a or b) are not completely identical with those of the U-turn structure expected from crystallographic study in terms of both the local conformation (e.g., $(\zeta, \alpha) = (g^-, t)$ in the tRNA U-turn but (g^+, g^+) or (g^+, t) in the turn structure of **1**) and the global conformation. (*vide infra*)

Similar computer simulations were carried out for the carbamate-linked dimer **2** using the same protocols and the program. Shown in Figure 7 are the lowest energy structures of **2** obtained from the calculation in which the carbamate linkage, N−C(=O)−O−C, was fixed in the trans conformation. The lowest energy values calculated were −1009.17, −1023.30, −1023.31, and −1035.81 kJ/mol for the structures in Figure 7a-d, respectively. The turn structure emerged when the conformations of both the 5′- and 3′-terminal riboses were fixed in the C3′-endo conformation (Figure 7a) and the backbone conformation was $(\zeta, \alpha) = (g^+, t)$. The other combinations of the 5′- and 3′-terminal conformations showed the stretch conformation.

The lowest energy structures of **2** in which the carbamate linkage is fixed in the cis conformation are shown in Figure 8. The energy values calculated were −1011.39, −1002.96, −1026.73, and −1026.79 kJ/mol for the structures in Figure 8a−d, respectively. Although the simultaneous fixation of the 5′- and 3′-terminal ribose in C3′ endo and C2′-endo conformations, respectively (Figure 8b), gave the turn structure, the γ of the 3′ terminal nucleotide was in the trans conformation, which is not identical with that of the nucleotide unit in the U-turn structure. All the other series of simulations uniformly gave stretch conformations.

To see which is a better U-turn mimic, **1** or **2**, compared with the 3D structures of **1** and **2** to the U-turn structure, the root mean square (rms) deviations between the natural U-turn structure and the cyclized dinucleoside monophosphates were calculated by the aid of the least-squares superimposition (for the detailed protocol, see the Experimental Section). Listed in Table 3 are the averaged values of the rms deviations of the low-energy structures (within 12 kJ/mol from the lowest energy

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Table 3. Root Mean Square Deviations (Å) of the Low-Energy Structures of the Cyclized Dimers Calculated from the Structures of UpU Having a U-Turn Conformation[a]

		compd **2**	
	compd **1**	trans	cis
5′:C3′-endo 3′:C3′-endo	4.15 (0.20)	3.87 (0.20)	4.22 (0.11)
5′:C3′-endo 3′:C2′-endo	4.26 (0.16)	4.53 (0.22)	3.86 (0.06)
5′:C2′-endo 3′:C3′-endo	4.40 (0.13)	5.05 (0.13)	5.13 (0.06)
5′:C2′-endo 3′:C2′-endo	4.45 (0.18)	4.60 (0.04)	4.30 (0.05)

[a] The standard deviations are shown in parentheses.

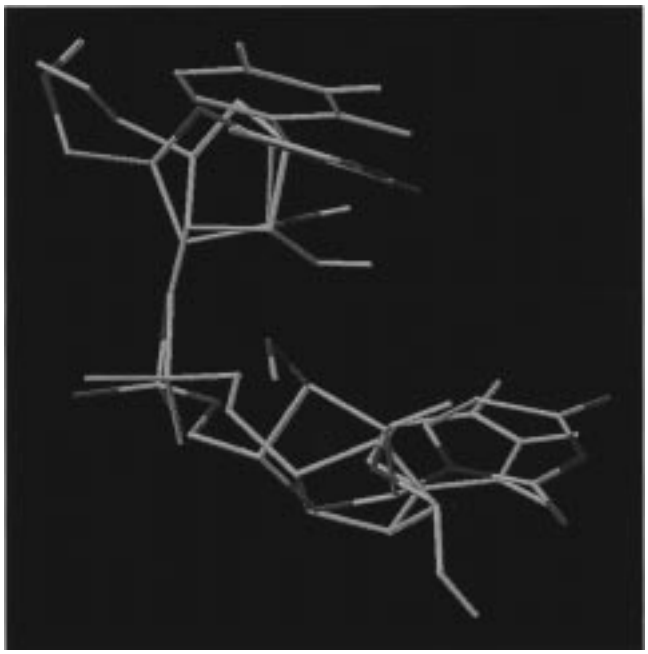

Figure 9. Superimposition of the structure of UpU (yellow) constructed by diplacement of G(34) of U(33)pG(34) dimer unit at the U-turn postition of $tRNA^{Phe}$ by uridine on the low-energy structure of **2** without the carbamoylmethyl group having the smallest rms deviation from the UpU described above.

structures). Table 3 shows that the cis carbamate-linked dimer of **2**, in which the 5′- and 3′-terminal riboses is C3′-endo and C2′-endo, respectively, has the smallest rms deviation. The trans carbamate-linked dimer of **2**, in which the conformations of both 5′- and 3′-terminal riboses are C3′-endo has a very similar rms deviation. The analysis of the averaged rms deviation suggested that the cis carbamate-linked conformer is a better U-turn mimic. A single structure that has the smallest rms deviation of 3.58 Å best fitting the natural U-turn of all trans and cis conformers of **2** is seen in the eighth lowest energy conformer (−1003.20 kJ/mol) of the trans structure having the 3′-endo conformation for both 5′- and 3′-terminal riboses. The backbone conformation of the lowest rms structure is $(\zeta, \alpha) = (g^-, t)$ with $\gamma = g^+$, which is identical with that in the natural U-turn structure. The superimposition of the lowest rms structure without the carbamoylmethyl group on the UpU fitted to the natural U-turn of $tRNA^{Phe}$ is shown in Figure 9, which shows reasonable agreement between the 3D structures of the turn structure of the carbamate linked dinucleoside monophosphate and the tRNA U-turn.

Conclusion

In conclusion, we have shown that the introduction of a cyclic structure, such as **1** and **2**, is effective for fixation of the conformation of dinucleoside monophosphates, which would be useful as turn structure models of functional RNAs. Between the amide-linked **1** and the carbamate-linked **2**, the latter is the better U-turn mimic as expected from the computational simulation. The rather large rms deviation of about 3.5 Å even in the best-fitted conformation of the carbamate linked **2**, however, suggested the U-turn structure in the anticodon loop on tRNA is stabilized not only by the interresidual hydrogen bonding, which is the focus of our present work, but also by other factors attributed to both the upstream and downstream strands of the U(33)pN(34). Therefore, the incorporation of the carbamate-linked dinucleoside monophosphate **2** into the anticodon loop or hammerhead ribozyme to explore the additive effect of the cyclic structure on the U-turn structure stability and the conformational changes of the U-turn region is an intriguing subject of study. It will also be interesting to incorporate cyclic structure **1**, which is less suitable as a U-turn mimic, into oligonucleotide duplexes because the stretch conformer of **1** (Figure 6c or d) apparently serves to extend the oligonucleotide chain toward both the 5′ and 3′ directions so that they can form bent duplexes. Conversion of **1** and **2** into the corresponding phosphoramidite units and further synthesis of oligonucleotides having a U-turn and a bend are now in progress and will be reported elsewhere.

Experimental Section

General Methods. ^1H, ^{13}C, and ^{31}P NMR spectra were recorded at 270, 68, and 109 MHz, respectively. The chemical shifts were measured from tetramethylsilane or DSS for ^1H NMR spectra, $CDCl_3$ (77 ppm) or DSS (0 ppm) for ^{13}C NMR spectra, and 85% phosphoric acid (0 ppm) for ^{31}P NMR spectra. The correct $^1H-^1H$ coupling constants were measured at 400 MHz. For measurement of the temperature dependence of ^{31}P NMR chemical shift, each sample (100 A_{260}) was dissolved in 10 mM cacodylate−1 mM EDTA (500 μL)−D_2O (100 μL) and the pH was adjusted to 7.1 by addition of 1 M NaOH. CD spectra were recorded on a spectrometer using a 0.5 cm cell. TLC was performed by the use of silica gel 60-F-254 (0.25 mm). Paper chromatography was performed by use of a descending technique with 3M papers using the solvent system 2-propanol−concentrated ammonia (25%)−water, 7:1:2, v/v/v. Column chromatography was performed with silica gel C-200, and a minipump for a goldfish bowl was conveniently used to attain sufficient pressure for rapid chromatographic separation. Reversed-phase column chromatography was performed by the use of μBondapak C-18 silica gel (prep S-500). Reversed-phase HPLC was performed on a LC module 1 using a μBondasphere C-18 column with a linear gradient starting from 0.1 M NH_4OAc, pH 7.0 and increasing CH_3CN at a flow rate of 1.0 mL/min for 30 min. Uridine was purchased from Yamasa Co., Ltd. UpU and UmpU were synthesized according to the usual phosphotriester method.[36] Snake venom phosphodiesterase and calf spleen phosphodiesterase were purchased from Boehringer Mannheim Biochemica Co., Ltd., and Nuclease P1 from Seikagaku Kougyou Co., Ltd. Pyridine was distilled from *p*-toluenesulfonyl chloride and then from calcium hydride and stored over molecular sieves 4A. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta.

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5-(Azidomethyl)-2′,3′-*O*-isopropylideneuridine (5). 5-(Hydroxymethyl)-2′,3′-*O*-isopropylideneuridine (**3**) (4.5 g, 14.3 mmol) was dissolved in dioxane (140 mL). Trimethylsilyl chloride (9.07 mL, 71.5 mmol) was added, and the mixture was stirred at 60 °C for 2.5 h. Solvent was removed under reduced pressure, and the residue was dissolved in DMF (140 mL). Sodium azide (5.58 g, 85.8 mmol) was added, and the mixture was stirred at 60 °C for 3 h. The resulting precipitate was filtered, and the filtrate was concentrated. The concentrate was extracted five times with CH_2Cl_2−pyridine (4:1, v/v), and the organic layer was concentrated under reduced pressure. The residue was chromatographed on a column of silica gel (CH_2Cl_2−MeOH, 100:1.5, v/v) gave **5** (2.41 g, 50%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.37 (3H, s, CH_3), 1.59 (3H, s, CH_3), 3.83 (1H, d, 5′-H_a, J_{gem} = 12.0 Hz), 3.95 (1H, dd, 5′-H_b, J_{gem} = 12.0 Hz, $J_{4',5'}$ = 2.4 Hz), 4.14 (1H, s, CH_2N_3), 4.81 (1H, dd, 4′-H, $J_{3',4'}$ = 3.0 Hz, $J_{4',5'}$ = 2.4 Hz), 4.96 (1H, dd, 3′-H, $J_{3',4'}$ = 3.0 Hz, $J_{2',3'}$ = 6.3 Hz), 5.02 (1H, dd, 2′-H, $J_{2',3'}$ = 6.3 Hz, $J_{1',2'}$ = 2.6 Hz), 5.65 (1H, d, 1′-H, J = 2.6 Hz), 7.51 (1H, s, 6-H), 9.05 (1H, br, NH); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 25.21, 27.19, 46.99, 62.55, 80.31, 83.79, 86.94, 95.60, 109.38, 114.47, 140.89, 150.03, 162.46; IR (KBr) 2100 cm^{-1}. Anal. Calcd for $C_{13}H_{17}N_5O_5\cdot H_2O$: C, 46.02; H, 5.05; N, 20.63. Found: C, 46.09; H, 4.97; N, 19.81.

5-(Aminomethyl)-2′,3′-*O*-isopropylideneuridine (7). Compound **5** (860 mg, 2.54 mmol) was rendered anhydrous by three coevaporations with dry pyridine and dissolved in dry pyridine (15 mL). Triphenylphosphine (1.07 g, 4.06 mmol) was added, and the resulting mixture was stirred at ambient temperature for 3 h. Aqueous ammonia (15 mL, 25%) was added, and this solution was stirred for an additional 3 h. The precipitate was filtered, and water was added to the filtrate. This mixture was washed two times with CH_2Cl_2, and the aqueous layer was concentrated under reduced pressure. The residue was charged on a C18 reversed-phase column. Elution with water gave **7** (567 mg, 71%). The presence of the amino group could be readily confirmed by the color identification test using the ninhydrin reagent, which showed a yellow spot on TLC: ^{1}H NMR (270 MHz, D_2O) δ 1.40 (3H, s, CH_3), 1.60 (3H, s, CH_3), 3.75 (1H, d, 5′-H_a, J_{gem} = 12.5 Hz), 3.86 (1H, d, 5′-H_b, J_{gem} = 12.5 Hz), 3.94 (1H, s, CH_2N), 4.40 (1H, m, 4′-H), 4.89 (1H, dd, 3′-H, $J_{3',4'}$ = 3.3 Hz, $J_{2',3'}$ = 6.6 Hz), 5.08 (1H, dd, 2′-H, $J_{2',3'}$ = 6.6 Hz, $J_{1',2'}$ = 2.6 Hz), 5.86 (1H, d, 1′-H, J = 2.6 Hz), 8.01 (1H, s, 6-H); ^{13}C NMR (67.8 MHz, D_2O) δ 27.06, 28.79, 40.25, 64.11, 83.10, 86.86, 89.06, 95.90, 111.90, 117.51, 143.92, 158.54, 173.72. Anal. Calcd for $C_{13}H_{19}N_3O_5\cdot 3/2H_2O$: C, 45.88; H, 6.22; N, 12.34. Found: C, 45.37; H, 6.15; N, 12.18.

2′-*O*-[(*tert*-Butoxycarbonyl)methyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*N*^{3}-(triphenylmethanesulfenyl)uridine (12). Compound **11** (1.88 g, 2.5 mmol) was rendered anhydrous by repeated coevaporation three times with dry pyridine followed by three coevaporations with dry toluene. The residue was dissolved in dry THF (25 mL). Sodium hydride (400 mg, 10 mmol) was added, and the mixture was stirred under argon. After 30 min, *tert*-butyl bromoacetate (1.6 mL, 10 mmol) was added, and the resulting mixture was stirred for 18 h. The mixture was quenched by addition of 200 mL of phosphate buffer (pH 7.0). After extraction with CH_2Cl_2, the organic layer was concentrated under reduced pressure. The residue was chromatographed on a column of silica gel with CH_2Cl_2−hexane (2:1, v/v) to give **12** (1.72 g, 78%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.03−1.09 (27H, m, *i*-Pr), 3.94 (1H, br, 5′-H_a), 3.96 (1H, m, 5′-H_b), 4.03−4.22 (5H, m, CH_2CO, 2′-H, 3′-H, 4′-H), 5.35 (1H, s, 1′-H), 5.48 (1H, d, 5-H, J = 8.1 Hz), 7.20−7.49 (15H, m, ArH), 7.63 (1H, d, 6-H, J = 8.1 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.29, 12.89, 12.94, 13.70, 16.82, 17.07, 17.25, 17.41, 28.11, 59.30, 74.89, 81.46, 81.55, 82.37, 89.51, 100.23, 100.54, 127.21, 127.39, 127.91, 130.01, 130.75, 137.45, 142.46, 142.61, 151.57, 163.07, 169.69. Anal. Calcd for $C_{46}H_{62}N_2O_9SSi_2$: C, 63.13; H, 7.14; N, 3.20. Found: C, 63.34; H, 7.05; N, 2.89.

2′-*O*-[(*tert*-Butoxycarbonyl)methyl]-*N*^{3}-(triphenylmethanesulfenyl)uridine (13). Compound **12** (1.72 g, 2.0 mmol) was dissolved in THF (20 mL), and tetrabutylammonium fluoride monohydrate (1.36 g, 4.3 mmol) and acetic acid (250 μL, 4.3 mmol) were added. The mixture was stirred at room temperature for 40 min. The solution was diluted with CH_2Cl_2 and washed with saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:1−100:1.5, v/v) to give **13** (1.23 g, 98%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.68 (9H, s, *t*-Bu), 4.00 (1H, d, 5′-H_a, J_{gem} = 11.9 Hz), 4.15 (1H, d, 5′-H_b, J_{gem} = 11.9 Hz), 4.19 (1H, d, CH_2CO, J_{gem} = 17.1 Hz), 4.21 (2H, m, 3′-H and 4′-H), 4.45 (1H, d, CH_2CO, J_{gem} = 17.1 Hz), 4.55 (1H, br, 2′-H), 5.46 (1H, d, 1′-H, $J_{1',2'}$ = 2.3 Hz), 5.72 (1H, d, 5-H, $J_{5,6}$ = 8.3 Hz), 7.41−7.66 (15H, m, ArH), 7.70 (1H, d, 6-H, $J_{5,6}$ = 8.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 28.07, 60.94, 67.80, 68.84, 74.95, 83.22, 84.10, 84.57, 91.84, 100.77, 127.33, 127.46, 127.92, 130.75, 132.02, 138.94, 142.35, 152.04, 162.82, 171.21. Anal. Calcd for $C_{34}H_{36}N_2O_8S\cdot 1/2H_2O$: C, 63.64; H, 5.81; N, 4.36. Found: C, 63.35; H, 5.86; N, 4.36.

2′-*O*-[(*tert*-Butoxycarbonyl)methyl]-5′-*O*-(4,4′-dimethoxytrityl)-*N*^{3}-(triphenylmethanesulfenyl)uridine (14). Compound **13** (1.23 g, 1.94 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (20 mL). Dimethoxytrityl chloride (723 mg, 2.10 mmol) was added, and the solution was stirred for 5 h. The mixture was partitioned with CH_2Cl_2−H_2O and extracted with saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was coevaporated three times with toluene and chromatographed on a column of silica gel with CH_2Cl_2−toluene (1:4, v/v) to give **14** (1.68 g, 88%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.53 (9H, s, *t*-Bu), 3.82 (7H, m, 5′-H_a, OCH_3), 4.05 (1H, m, 4′-H), 4.16 and 4.32 (2H each, d, CH_2CO, J_{gem} = 18.0 Hz), 4.29 (2H, m, 2′-H and 3′-H), 5.16 (1H, d, 5-H, $J_{5,6}$ = 8.3 Hz), 5.38 (1H, s, 1′-H), 6.85−7.52 (23H, m, ArH), 7.82 (1H, d, 6-H, $J_{5,6}$ = 8.3 Hz); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 28.02, 55.17, 60.59, 67.35, 68.25, 74.93, 82.95, 83.07, 84.80, 86.78, 89.76, 100.49, 113.19, 123.65, 127.03, 127.26, 127.39, 127.91, 128.05, 129.99, 130.14, 130.71, 135.13, 135.35, 135.87, 137.47, 142.46, 144.49, 149.78, 151.82, 158.58, 158.63, 162.82, 171.05. Anal. Calcd for $C_{55}H_{54}N_2O_{10}S$: C, 70.65; H, 5.82; N, 2.99. Found: C, 70.74; H, 5.82; N, 2.85.

2′-*O*-[(*tert*-Butoxycarbonyl)methyl]-5′-*O*-(4,4′-dimethoxytrityl)uridine (15). Compound **14** (464 mg, 0.50 mmol) was dissolved in toluene (5 mL) and heated to 120 °C. Tributyltin hydride (340 μL, 1.3 mmol) was added and the mixture stirred for 5 min in the presence of azabisisobutyronitrile (2 mg). The mixture was cooled with water and chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:1, v/v) to give **15** (379 mg, 78%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.49 (9H, s, *t*-Bu), 3.54 (2H, m, 5′-H), 3.80 (6H, s, OCH_3), 3.95 (1H, d, 2′-H J = 4.9 Hz), 4.25 and 4.37 (2H each, d, CH_2CO, J_{gem} = 17.0 Hz), 4.15 (2H, m, 4′-H, 3′-OH), 4.46 (1H, dd, 3′-H), 5.26 (1H, d, 5-H, $J_{5,6}$ = 8.2 Hz), 5.87 (1H, s, 1′-H), 6.82−7.40 (13H, m, ArH), 8.03 (1H, d, 6-H, $J_{5,6}$ = 8.2 Hz), 8.56 (1H, br, N^3-H): ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 28.03, 55.23, 61.13, 68.00, 68.57, 83.15, 83.36, 85.27, 86.97, 88.43, 101.89, 113.30, 128.00, 128.12, 130.06, 130.19, 135.09, 135.35, 139.95, 144.42, 149.97, 158.65, 158.71, 162.93, 170.67. Anal. Calcd for $C_{36}H_{40}N_2O_{10}\cdot 2H_2O$: C, 62.06; H, 6.36; N, 4.01. Found: C, 62.24; H, 5.81; N, 4.00.

3′-*O*-(*tert*-Butyldimethylsilyl)-2′-*O*-(carboxymethyl)-5′-*O*-(4,4′-dimethoxytrityl)uridine (17). Compound **15** (226 mg, 0.34 mmol) was dissolved in 0.2 M KOH−pyridine (5 mL:5 mL). After being stirred for 1.5 h, the mixture was neutralized with Dowex 50W × 8 (pyridinium form) and filtered. The filtrate was concentrated and partitioned between ether and H_2O. The aqueous layer was washed twice with ether, evaporated under reduced pressure, and rendered anhydrous by repeated coevaporation with dry pyridine. The residue was further coevaporated with dry toluene and finally dissolved in DMF (1 mL). *tert*-Butyldimethylsilyl chloride (150 mg, 1.0 mmol) and imidazole (68 mg, 1.0 mmol) were added. The solution was stirred at room temperature for 60 h and then at 40 °C for 30 min. The mixture was diluted with CH_2Cl_2−pyridine (2:1, v/v) and washed three times with saturated

$NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:5, v/v) to give **17** (379 mg, 78%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.67 and 0.66 (6H each, s, CH_3), 0.79 (9H, s, *t*-Bu), 3.34 (1H, d, 5′-H, J_{gem} = 9.2 Hz), 3.79 (7H, m, OCH_3, 5′-H), 4.06 (1H, d, 2′-H J = 2.7 Hz), 4.18 (1H, m, 4′-H), 4.28 (12H, m, CH_2CO, 3′-H), 4.44 (1H, d, CH_2CO, J_{gem} = 16.2 Hz), 6.10 (1H, s, 1′-H), 5.29 (1H, d, 5-H, $J_{5,6}$ = 7.9 Hz), 6.82−7.36 (13H, m, ArH), 8.20 (1H, d, 6-H, $J_{5,6}$ = 7.9 Hz), 10.29 (1H, br, N^3-H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.16, −4.56, 17.92, 25.55, 55.20, 68.07, 69.36, 82.80, 82.97, 102.18, 113.15, 113.21, 125.23, 127.21, 127.91, 128.16, 128.34, 128.97, 130.24, 134.90, 134.99, 140.54, 143.92, 150.82, 158.72, 164.26, 173.40. Anal. Calcd for $C_{38}H_{45}N_2O_{10}Si\cdot 1/2H_2O$: C, 62.71; H, 6.51; N, 3.85. Found: C, 62.68; H, 6.71; N, 3.84.

Amide-Linked Diuridine Dimer 19. Compound **17** (222 mg, 0.28 mmol) and *N*-hydroxysuccinimide (38 mg, 0.34 mmol) were dissolved in DMF (2 mL). *N*-Ethyl-*N*′-(dimethylamino)carbodiimide hydrochloride (63 mg, 0.34 mmol) was added, and the mixture was stirred for 6 h. Compound **7** (107 mg, 0.34 mol) and triethylamine (47 μL, 0.34 mmol) were added, and the resulting solution was stirred for another 2 h. The mixture was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 extract was washed three times with saturated $NaHCO_3$ and five times with water. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was evaporated to dryness under reduced pressure and chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:2, v/v) to give **19** (312 mg, 93%): ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.12 and 0.02 (3H each, s, CH_3), 0.77 (9H, s, *t*-Bu), 1.34 and 1.57 (3H each, s, isopropylidene), 3.32 (1H, d, 5′-H_{a1}, J_{gem} = 9.2 Hz), 3.72−3.85 (9H, m, 2′-H_a, 5′-H_{b1}, OCH_3, 5′-H_{a2}), 3.95 (1H, d, 5′-H_{b2}, J_{gem} = 12.2 Hz), 4.01−4.16 (3H, m, 4′-H_a, CH_2N), 4.19 (2H, s, CH_2CO), 4.28 (1H, m, 3′-H_a), 4.89 (2H, br, 2′-H_b, 3′-H_b), 5.30 (1H, d, 5-H_a, $J_{5,6}$ = 7.9 Hz), 5.80 (1H, s, 1′-H_b), 5.95 (1H, d, 1′-Ha, $J_{1',2'}$ = 1.2 Hz), 6.82−7.35 (14H, m, ArH, 6-H_b), 7.55 (1H, t, NHCO), 8.05 (1H, d, 6-H_a, $J_{5,6}$ = 7.9 Hz), 9.60 and 9.90 (1H each, br, N^3-H). Anal. Calcd for $C_{51}H_{63}N_5O_{15}Si\cdot H_2O$: C, 59.35; H, 6.15; N, 6.78. Found: C, 59.59; H, 6.34; N, 6.67.

Phosphorylated Diuridine Dimer 20. Compound **19** (128 mg, 0.13 mmol), cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate (99 mg, 0.26 mmol), and 1*H*-tetrazole (36 mg, 0.52 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and dissolved in dry pyridine (2 mL). DDS (129 mg, 0.39 mmol) was added. The mixture was stirred at room temperature for 30 min and partitioned between CH_2Cl_2 and H_2O. The organic layer was washed three times with saturated $NaHCO_3$, dried over Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure. The residue was coevaporated with toluene and chromatographed on a column of silica gel with CH_2Cl_2−MeOH to give **20** (135 mg, 82%): ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.13 and 0.01 (3H each, s, CH_3), 0.76 (9H, s, *t*-Bu), 1.34 and 1.57 (3H each, s, isopropylidene), 3.32 and 3.73 (1H each, d, 5′-H_a, J_{gem} = 9.2 Hz), 3.79 (6H, s, OCH_3), 3.83 (1H, dd, 2′-H_a, $J_{2',3'}$ = 4.5 Hz, $J_{1',2'}$ = 1.7 Hz), 3.97−4.30 (6H, m, CH_2N, CH_2CO, 4′-H_a, 3′-H_a), 4.35 (1H, m, 4′-H_b), 4.43 (2H, m, 5′-H_b), 4.75 (1H, dd, 3′-H_b, $J_{2',3'}$ = 6.3 Hz, $J_{3',4'}$ = 3.3 Hz), 4.92 (1H, d, 2′-H_b, $J_{2',3'}$ = 6.3 Hz, $J_{1',2'}$ = 2.3 Hz), 5.29 (1H, d, 5-H_a, J_{gem} = 8.2 Hz), 5.77 (1H, d, 1′-H_b, $J_{1',2'}$ = 2.3 Hz), 5.94 (1H, d, 1′-Ha, $J_{1',2'}$ = 1.7 Hz), 6.82−7.56 (24H, m, ArH, 6-H_b), 8.04 (1H, d, 6-H_a, $J_{5,6}$ = 8.2 Hz), 9.32 and 9.65 (1H each, br, N^3-H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ −5.21, −4.49, 17.81, 25.09, 25.48, 27.05, 35.83, 55.22, 60.63, 62.46, 69.51, 70.12, 80.88, 82.82, 83.72, 85.39, 87.10, 87.78, 88.12, 94.59, 102.46, 109.76, 113.19, 113.23, 113.59, 125.25, 127.28, 127.94, 128.18, 128.30, 128.99, 130.21, 134.81, 134.90, 139.30, 141.04, 143.85, 150.19, 150.60, 158.76, 163.46, 163.56, 170.55; ^{31}P NMR (109 MHz, $CDCl_3$) δ 50.7 ppm. Anal. Calcd for $C_{67}H_{72}N_5O_{16}SiS_2P\cdot 2H_2O$: C, 65.14; H, 5.22; N, 2.26. Found: C, 65.30; H, 5.45; N, 2.69.

The Fully Protected Amide-Linked Diuridine Monophosphate 22. Compound **20** (122 mg, 95 μmol) was dissolved in triethylamine−pyridine−H_2O (2 mL:2 mL:1.5 mL) and stirred at 40 °C for 1 h. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in THF (6 mL), and tetrabutylammonium fluoride monohydrochloride (62 mg, 0.24 mmol) was added. The solution was stirred for 3 h and evaporated to dryness under reduced pressure. The residue was dissolved in H_2O−pyridine (6 mL, 1:1, v/v) and chromatographed on a Dowex 50W × 8 column (pyridinium form, 16 mL) with H_2O−pyridine (100 mL, 1:1, v/v). The fraction was rendered anhydrous by repeated coevaporation with dry pyridine, and the residue was dissolved in dry pyridine (5 mL). DDS (94 mg, 0.29 mmol) was added. The mixture was stirred at room temperature for 1.5 h and diluted with CH_2Cl_2. The solution was washed three times with saturated $NaHCO_3$. The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was coevaporated with toluene and chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:2.5, v/v) to give **22** (51 mg, 50%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.33 and 1.58 (3H each, s, isopropylidene), 3.22 and 3.57 (1H each, m, 5′-H_a), 3.80 (6H, s, OCH_3), 4.01−4.10 (3H, m, CH_2N, 4′-H_a), 4.12−4.29 (3H, m, 2′-H_a, CH_2CO), 4.41−4.50 (3H, m, 4′-H_b, 5′-H_b), 4.53 (1H, dd, 3′-H_b, $J_{2',3'}$ = 6.3 Hz, $J_{3',4'}$ = 3.0 Hz), 4.62 (1H, dd, 2′-H_b, $J_{2',3'}$ = 6.3 Hz, $J_{1',2'}$ = 2.3 Hz), 4.92 (1H, m, 3′-H_a), 5.30 (1H, d, 5-H_a, J_{gem} = 8.2 Hz), 5.97 (1H, d, 1′-H_b, $J_{1',2'}$ = 2.3 Hz), 6.09 (1H, d, 1′-Ha, $J_{1',2'}$ = 5.6 Hz), 6.82−7.64 (19H, m, ArH, 6-H_b), 7.67 (1H, d, 6-H_a, J = 8.2 Hz), 8.89, 9.07 (1H, 1H, br, br, N^3-H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 25.30, 27.08, 55.28, 61.89, 67.80,70.14, 79.32, 81.42, 81.74, 84.31, 84.48, 85.46, 86.15, 91.45, 103.38, 111.30, 113.41, 114.45, 124.17, 124.28, 127.48, 128.12, 129.79, 130.21, 134.50, 134.57, 134.86, 134.93, 138.81, 143.65, 150.21, 150.80, 158.90, 162.50, 162.75, 168.54; ^{31}P NMR (109 MHz, $CDCl_3$) δ 25.5, 24.7 ppm. Anal. Calcd for $C_{51}H_{52}N_5O_{16}SP\cdot 2H_2O$: C, 56.19; H, 5.18; N, 6.42. Found: C, 56.21; H, 5.18; N, 6.67.

Amide-Linked Diuridine Monophosphate 1. Compound **22** (39 mg, 36.5 μmol) was dissolved in pyridine (2 mL) and bis(tributyltin) oxide (370 μL, 0.7 mmol). After 3 h, trimethylsilyl chloride (140 μL, 1.1 mmol) was added, and the mixture was stirred for 5 min. The solution was diluted with H_2O−pyridine (1:1) and washed three times with hexane. The aqueous layer was extracted with CH_2Cl_2, and the extract was dried over Na_2SO_4 and filtered. The filtrate was evaporated, and the remaining pyridine was completely removed by repeated coevaporation with H_2O. The residue was dissolved in 60% formic acid (10 mL) and stirred at room temperature for 20 h. The solution was evaporated under reduced pressure, and the residue was chromatographed on paper (Whatman 3MM) with ^{i}PrOH-concentrated ammonia (25%)−H_2O (7:1:2, v/v/v) to give **1** (438 A_{260}, 61%): ^{1}H NMR (270 MHz, D_2O) δ 3.81 (1H, dd, 5′-H_a, J_{gem} = 13.2 Hz, $J_{4',5''}$ = 3.8 Hz), 3.93 (1H, dd, 5″-H_a, J_{gem} = 13.2 Hz, $J_{4',5''}$ = 2.8 Hz), 4.2−4.3 (8H, m, CH_2N, 4′-H_a, 2′-H_b, 3′-H_b, 4′-H_b, 5′-H_b), 4.35 (3H, m, 4′-H_a, CH_2CO), 4.40 (1H, t, 2′-H_a, $J_{1',2'}$ = $J_{2',3'}$ = 4.9 Hz), 4.78 (1H, ddd, 3′-H_a, $J_{2',3'}$ = 4.3 Hz, $J_{3',4'}$ = 2.8 Hz, $J_{3',P}$ = 7.8 Hz), 5.88 (1H, d, 5-H_a, J_{gem} = 7.9 Hz), 6.00 (1H, d, 1′-H_b, $J_{1',2'}$ = 3.9 Hz), 6.07 (1H, d, 1′-Ha, $J_{1',2'}$ = 4.6 Hz), 7.52 (1H, s, 6-H_b), 7.87 (1H, d, 6-H_a, $J_{5,6}$ = 7.9 Hz); ^{13}C NMR (67.8 MHz, D_2O) δ 35.24, 61.06, 65.87, 69.48, 70.26, 72.23, 74.86, 81.08, 83.59, 83.73, 84.53, 88.48, 89.07, 103.39, 112.26, 136.26, 142.31, 172.68; ^{31}P NMR (109 MHz, D_2O) δ 0.61 ppm.

2′-*O*-[(4-Nitrophenoxy)carbonyl]-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-di-yl)uridine (23). Compound **10** (4.5 g, 9.3 mmol) was dissolved in dry toluene (100 mL). 4-Nitrophenyl chloroformate (2.2 g, 11.1 mmol) and pyridine (840 μL, 11.1 mmol) were added, and the resulting mixture was stirred at room temperature for 1 h. The solution was dissolved with ether (300 mL) and washed three times with saturated $NaHCO_3$ (300 mL). The ether layer was dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:1, v/v) to give **23** (5.8 g, 96%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.02−1.11 (27H, m, *i*-Pr), 4.00−4.14 (2H, m, 5″-H, 4′-H), 4.27 (1H, d, 5′-H, J_{gem} = 13.5 Hz), 4.46 (1H, dd, 3′-H_b, $J_{2',3'}$ = 4.6 Hz, $J_{3',4'}$ = 9.2 Hz), 5.33 (1H, d, 2′-H, $J_{2',3'}$ = 4.6 Hz), 5.75 (1H, d, 5-H, $J_{5,6}$ = 7.9 Hz), 5.96 (1H, s, 1′-H), 7.40 (2H, d, Ar-H, J = 9.2 Hz), 7.76 (1H, d,

6-H, $J_{5,6}$ = 7.9 Hz), 8.28 (2H, d, Ar-H, J = 9.2 Hz), 9.73 (1H, br, 3-H); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.64, 12.80, 13.32, 16.64, 16.77, 16.89, 17.13, 17.16, 17.25, 17.33, 59.21, 64.62, 67.67, 80.10, 81.92, 87.87, 102.37, 121.64, 125.30, 138.90, 145.46, 149.92, 151.09, 155.35, 163.32. Anal. Calcd for $C_{28}H_{41}N_3O_4Si_2$: C, 51.60; H, 6.34; N, 6.44. Found: C, 51.54; H, 6.28; N, 6.38.

Carbamate-Linked Diuridine Dimer 24. A mixture of **23** (521 mg, 0.80 mmol) and **7** (275.7 mg, 0.88 mol) in DMF (3 mL) was stirred for 1 min and then partitioned between CH_2Cl_2 (50 mL) and saturated $NaHCO_3$ (50 mL). The aqueous layer was extracted five times with CH_2Cl_2 (50 mL), and the organic layer was collected, dried over Na_2SO_4, and filtered. After being evaporated to dryness, the residue was chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:2.5, v/v) to give **24** (587 mg, 89%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.92−1.25 (27H, m, *i*-Pr), 1.36 and 1.58 (3H each, s, CH_3), 3.64−3.99 (6H, m, 5′-H_{a1}, 5′-H_{b1}, 5′-H_{b2}, CH_2N, 4′-H_a), 4.19 (1H, d, 5′-H_{a2}, J_{gem} = 12.2 Hz), 4.29 (1H, m, 3′-H_a), 4.45 (1H, m, 4′-H_b), 4.87−4.94 (2H, m, 2′-H_b, 3′-H_b), 5.17 (1H, d, 2′-H_a, $J_{2',3'}$ = 4.9 Hz), 5.69 (1H, d, 5-H_a, $J_{5,6}$ = 7.9 Hz), 5.77 (2H, m, 1′-H_a, 1′-H_b), 5.87 (1H, t, OCONH, J = 5.8 Hz), 7.66 (1H, d, 6-H_a, $J_{5,6}$ = 7.9 Hz), 7.85 (1H, s, 6-H_b), 9.20 (2H, br, 3-H_z, 3-H_b); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.53, 12.81, 13.35, 16.71, 16.79, 17.18, 17.22, 17.3, 17.36, 25.09, 26.99, 37.92, 59.55, 62.36, 67.75, 76.07, 80.90, 82.00, 85.45, 87.78, 88.46, 94.88, 102.21, 109.90, 113.55, 139.17, 140.75, 150.08, 150.24, 155.44, 163.56, 164.11. Anal. Calcd for $C_{35}H_{55}N_5O_4Si_2 \cdot 2H_2O$: C, 48.77; H, 6.90; N, 8.12. Found: C, 48.55; H, 7.02; N, 8.34.

Acetylation of 24. Compound **24** (1.15 g, 1.4 mmol) was dissolved in pyridine (10 mL), and acetic anhydride (520 μL, 5.6 mmol) was added. The resulting solution was stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed three times with saturated $NaHCO_3$ (100 mL). The organic layer was collected, dried over Na_2SO_4, evaporated to dryness by repeated coevaporation with toluene. The residue was chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:1.8, v/v) to give **25** (1.2 g, 99%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.92−1.25 (27H, m, *i*-Pr), 1.56, 1.34 (3H, 3H, s, s, CH_3), 2.07 (3H, s, CH_3CO), 3.92−4.30 (9H, m, 3′-H_a, 4′-H_a, 4′-H_b 5′-H_a, 5′-H_b, CH_2N), 4.98 (1H, m, 3′-H_b), 4.97 (1H, d, 2′-H_b, $J_{2',3'}$ = 6.3 Hz), 5.20 (1H, d, 2′-H_a, $J_{2',3'}$ = 5.3 Hz), 5.66 (1H, d, 5-H_a, $J_{5,6}$ = 8.2 Hz), 5.76 (1H, s, 1′-H_b), 5.81 (2H, m, 1′-H_a, NHCO), 7.48 (1H, s 6-H_b), 7.61 (1H, d, 6-H_b, $J_{5,6}$ = 8.2 Hz), 9.48 and 9.73 (1H each, br, 3-H_a and 3-H_b); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 12.42, 12.64, 12.72, 13.26, 16.66, 17.06, 17.11, 17.16, 17.25, 20.65, 25.12, 26.94, 37.56, 59.53, 64.15, 67.64, 75.80, 81.10, 81.87, 84.35, 85.09, 88.57, 94.43, 102.00, 111.12, 114.36, 139.10, 141.11, 149.85, 149.97, 154.90, 163.50, 163.95, 170.48. Anal. Calcd for $C_{37}H_{57}N_5O_{15}Si_2 \cdot 2H_2O$: C, 49.43; H, 6.84; N, 7.79. Found: C, 49.10; H, 6.77; N, 7.86.

Diol Derivative 26. Compound **25** (1.12 g, 1.29 mmol) was dissolved in THF (12 mL), and tetrabutylammonium fluoride monohydrate (900 mg, 2.84 mol) and acetic acid (160 μL, 2.84 mmol) were added. The resulting solution was stirred at room temperature for 40 min. The mixture was diluted with CH_2Cl_2−pyridine (60 mL:30 mL) and washed with saturated $NaHCO_3$ (100 mL). The aqueous layer was back-extracted with CH_2Cl_2−pyridine (60 mL:30 mL). The conbined organic layers were dried over Na_2SO_4, filtered, and evaporated by repeated coevaporation with toluene. The residue was chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:5.5, v/v) to give **26** (770 mg, 96%): ^{1}H NMR (270 MHz, $CDCl_3$−5% CD_3OD) δ 1.29 and 1.53 (3H, 3H, s, s, CH_3), 1.97 (3H, s, $CH_3C{=}O$), 3.76 (2H, m, 5′-H_a), 4.15−4.28 (6H, m, 3′-H_a, 4′-H_a, 5′-H_b, CH_2N), 4.74 (1H, m, 3′-H_b), 5.02 (2H, m, 2′-H_a, 2′-H_b), 5.62 (1H, d, 5-H_a, $J_{5,6}$ = 7.9 Hz), 5.72 (1H, d, 1′-H_b, $J_{1',2'}$ = 1.7 Hz), 5.94 (1H, d, 1′-H_a, $J_{1',2'}$ = 5.9 Hz), 7.31 (1H, s, 6-H_b), 7.82 (1H, d, 6-H_b, $J_{5,6}$ = 7.9 Hz), 7.90 and 8.55 (1H each, br, 3-H_a and 3-H_b); ^{13}C NMR (67.8 MHz, CD_3OD) δ 20.75, 25.50, 27.40, 48.05, 62.35, 65.46, 70.92, 77.48, 82.84, 85.71, 86.52, 86.93, 88.84, 96.06, 102.98, 112.27, 115.40, 141.92, 142.71, 151.78, 152.25, 157.41, 165.31, 165.96, 172.43. Anal. Calcd for $C_{25}H_{31}N_5O$: C, 48.00; H, 5.00; N, 11.19. Found: C, 47.71; H, 5.25; N, 10.97.

Phosphorylated Carbamate-Linked Diuridine Dimer 27. Compound **26** (694 g, 1.11 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and dissolved in dry pyridine (10 mL). 4,4′-Dimethoxytrityl chloride (488 mg, 1.44 mmol) was added, and the resulting mixture was stirred at room temperature. After 4 h, cyclohexylammonium *S*,*S*-diphenyl phosphorodithioate (677 mg, 1.78 mmol), 1*H*-tetrazole (310 mg, 4.43 mmol), and DDS (2.01 g, 6.66 mmol) were added. The solution was stirred for 3.5 h and then extracted between CH_2Cl_2−water (50 mL:50 mL). The organic layer was washed three times with saturated $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and evaporated to dryness by repeated coevaporation with toluene. The residue was chromatographed on a column of silica gel with CH_2Cl_2−MeOH (100:1.5, v/v) to give **27** (1.15 g, 88%). The fraction obtained was an equilibrium mixture of the two rotamers at the carbamate bond, which gave two distinguishable set of signals in NMR spectra in $CDCl_3$: ^{1}H NMR (270 MHz, $CDCl_3$) major isomer δ 1.20, 1.58 (3H, 3H, s, s, CH_3), 2.01 (3H, s, CH_3CO), 3.33 (2H, m, 5″-H_a), 3.77 (7H, m, OCH_3, 5′-H_a), 4.08 (1H, br, 4′-H_a), 4.20−4.50 (5H, m, 4′-H_b, 5′-H_b, 5″-H_b, CH_2N), 4.88 (1H, m, 3′-H_b), 5.11−5.31 (2H, m, 2′-H_a, 3′-H_a), 5.40 (1H, d, 5-H_a, $J_{5,6}$ = 7.3 Hz), 5.45 (1H, m, 2′-H_a), 5.88 (1H, s, 1′-H_b), 6.17 (1H, d, 1′-H_a, $J_{1',2'}$ = 7.3 Hz), 6.83−7.65 (15H, m, 6-H_a, 6-H_bArH), 7.90 and 8.55 (1H each, br, 3-H_a and 3-H_b); ^{31}P NMR (109 MHz, $CDCl_3$) δ 50.00; minor isomer δ 1.50 and 1.63 (3H each, s, CH_3), 3,45 (1H, m, 5′-H_a), 5.00 (1H, m, 3′-H_b), 6.02 (1H, s, 1′-H_b), 6.41 (1H, d, 1′-H_a, $J_{1',2'}$ = 7.9 Hz), 9.05 and 9.69 (1H each, br, 3-H_a and 3-H_b); ^{31}P NMR (109 MHz, $CDCl_3$) δ 50.18; ^{13}C NMR (67.8 MHz, DMSO-d_6) δ 20.72, 25.09, 26.85, 53.35, 55.17, 62.82, 64.31, 73.96, 81.53, 83.52, 84.26, 85.10, 85.73, 87.60, 96.05, 103.02, 110.71, 113.35, 114.68, 115.00, 125.52, 125.62, 125.71, 127.21, 128.03, 128.07, 128.12, 129.45, 129.52, 129.56, 129.72, 129.83, 130.01, 130.06, 130.10, 134.56, 135.78, 138.96, 139.12, 140.97, 143.52, 143.68, 149.72, 142.79, 150.91, 150.96, 154.50, 158.62, 158.71, 162.50, 163.45, 164.10, 170.57. Anal. Calcd for $C_{58}H_{58}N_5O_{17}PS_2$: C, 58.43; H, 4.90; N, 5.87. Found: C, 58.08; H, 4.94; N, 5.69.

The Fully Protected Carbamate-Linked Diuridine Monophosphate 29. Compound **27** (204 mg, 0.17 mmol) was dissolved in 0.2 N KOH−pyridine (10 mL:10 mL) and stirred for 40 min at room temperature. The mixture was passed through a Dowex 50W × 8 column (pyridinium form, 10 mL), and elution was performed with H_2O−pyridine (1:1, v/v, 100 mL). Triethylamine (5 mL) was added, and the eluent was evaporated to dryness under reduced pressure. The residue was rendered anhydrous together with 1*H*-tetrazole (60 mg, 0.85 mmol) by repeated coevaporation with pyridine and finally dissolved in dry pyridine (2 mL). To this solution was added DDS (169 mg, 0.51 mmol), and the resulting mixture was stirred at room temperature for 40 min. The solution was diluted with ether (50 mL) and washed three times with saturated $NaHCO_3$ (50 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated to dryness by repeated coevaporation with toluene. The residue was chromatographed on a column of silica gel with CH_2Cl_2:MeOH (100:2.5, v/v) to give **29** (87 mg, 49%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.30 and 1.52 (3H each, s, CH_3), 3.46 (2H, br, 5′-H_a), 3.78 and 3.79 (6H each, s, OCH_3), 3.92−4.37 (5H, m, 5′-H_b, 4′-Hb, CH_2), 4.53−4.79 (2H, m, 2′-H_b, 4′-H_a), 4.83 (1H, m, 3′-H_b), 5.13 (1H, dd, 3′-H_a, J = 4.1 Hz, J = 9.2 Hz), 5.44−5.63 (2H, m, 2′-H_a, 5-H), 6.17 (1H, d, 1′-H_a, $J_{1',2'}$ = 8.2 Hz), 6.29 (1H, d, 1′-H_b, $J_{1',2'}$ = 2.0 Hz), 6.83−7.72 (17H, m, ArH, 6-H_a, 6-H_b), 10.03 and 10.21 (1H each, br, 3-H_a and 3-H_b); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 25.32, 25.45, 27.12, 38.44, 38.69, 55.24, 62.98, 68.11, 68.10, 78.08, 78.64, 82.91, 83.11, 84.10, 84.87, 85.39, 87.62, 87.75, 88.97, 89.09, 103.38, 111.52, 111.70, 113.39, 113.46, 114.99, 115.38, 123.72, 123.86, 127.22, 128.05, 128.09, 128.16, 128.75, 129.72, 129.99, 130.05, 130.84, 134.72, 134.86, 134.99, 135.06, 135.33, 135.42, 135.60, 137.43, 138.69, 143.65, 149.60, 149.88, 151.48, 151.73, 154.75, 154.93, 158.72, 158.78, 162.82, 162.96, 163.83; ^{31}P NMR (109 MHz, D_2O) δ 26.15, 20.48 ppm.

Anal. Calcd for $C_{50}H_{50}N_5O_{16}PS\cdot 7/2H_2O$: C, 54.45; H, 5.21; N, 6.35. Found: C, 54.49; H, 4.82; N, 6.18.

Carbamate-Linked Diuridine Monophosphate 2. Compound **29** (41 mg, 40 μmol) was dissolved in pyridine (2 mL). Bis(tributyltin) oxide (410 μL, 0.80 mmol) was added, and the resulting mixture was stirred for 3 h at ambient temperature. Trimethylsilyl chloride was added, and then the solution was diluted with H_2O−pyridine (10 mL:10 mL). After being washed three times with hexane (20 mL), the mixture was extracted three times with CH_2Cl_2. The CH_2Cl_2 extracts were collected, dried over Na_2SO_4, filtered, and coevaporated five times with water to remove pyridine completely. The residue was dissolved in 60% formic acid (10 mL), and the solution was stirred for 20 h. The formic acid was removed from the mixture by repeated coevaporation with water. The resulting residue was chromatographed on Whatman 3MM paper with *i*-PrOH−concentrated ammonia−H_2O (7:1:2, v/v/v) to give **29** (459 A_{260}, 58% assuming that the ϵ value of **29** was same as that of UpU): ^{1}H NMR (270 MHz, D_2O) of the major equilibrium isomer δ 3.81−3.86 (3H, m, 5″-H_b and 5′-H_a), 3.92 (1H, br, 5′-H_b), 4.05−4.10 (3H, m, 5′-H_a, CH_2), 4.17−4.20 (2H, m, 2′-H_b and 4′-H_b), 4.27−4.39 (2H, m, 3′-H_b and 4′-H_a), 4.75 (1H, m, 3′-H_a), 5.30 (1H, m, 2′-H_a), 5.85 (1H, d, 5-H_a, $J_{5,6}$ = 7.9 Hz), 6.00−6.03 (2H, m, 1′-H_a and 1′-H_b, $J_{1'a,2'a}$ = 8.0 Hz, $J_{1'b,2'b}$ = 5.1 Hz), 7.80 (1H, d, 6-H_a, $J_{5,6}$ = 7.9 Hz), 7.89 (1H, s, 6-H_b); ^{1}H NMR (270 MHz, D_2O) of the minor equilibrium isomer δ 5.38 (m, 2′-H_a), 6.12 (d, 1′-H_a, $J_{1',2'}$ = 4.5 Hz), 7.38 (s, 6′-H_b); ^{13}C NMR (67.8 MHz, D_2O) δ 30.13, 38.13, 60.30, 61.04, 63.97, 65.57, 68.69, 70.38, 71.73, 73.19, 73.54, 74.01, 74.48, 82.62, 84.09, 84.94, 85.46, 87.76, 88.06, 102.60, 112.02, 134.73, 139.17, 141.31, 151.35, 151.78, 156.15, 165.10, 165.87, 166.01; ^{31}P NMR (109 MHz, D_2O) δ 0.86, 0.32 ppm.

CD Spectra. Dinucleoside monophosphate was dissolved in 10 mM sodium phosphate pH 7.0 (2 mL), and the absorbance was adjusted to 0.5A_{260nm}. The spectra were accumulated 16 times and then averaged for smoothing.

400 MHz NMR Spectra. Dinucleoside monophosphate (100A_{260nm}) was dissolved in 10 mM sodium phosphate pH 7.0 (600 μL) and then lyophylized three times with 99.8% D_2O and finally redissolved in 99.95% D_2O (600 μL). The spectra were recorded at 400 MHz, and the coupling constants were simulated by using the "Deconvolusion" option compiled in the spectrometer.

Enzymatic Digestion. The cyclized dinucleoside monophosphate was dissolved in a buffer of 50 mM Tris−HCl pH 8.0 for SVP, 20 mM sodium acetate−0.1 mM $ZnCl_2$ for NP1, or 200 mM ammonium acetate pH 5.7 for CSP and incubated at 35 °C. The reactions were analyzed by using reversed-phase HPLC.

Computational Methods. The four initial structures of **1** and **2** each were constructed by using the structure of uridine or thymidine residues implemented in Macro Model ver. 4.5. The ribose moieties were fixed in the conformation of the initial structure by applying the constraints on all five endocyclic torsions of the riboses. The four initial structures in which the 5′-terminal ribose and 3′-terminal riboses were fixed in C3′-endo or C2′-endo conformations were simulated independently in the following calculation. The force field used was the AMBER* by modifying the distance dependent dielectric constant to the constant dielectric. The effect of the solvent was included by using the GB/SA solvent model implemented in the software. The default values for the atomic partial charges were used in all the calculations. The initial structures were energy minimized, and the structures thus obtained were used as starting structures for the next simulations. The local minima were searched by the 500 step energy minimization of 5000 structures generated by using the MCMM option. The low-energy structure (within 20 kJ/mol from the lowest energy structures) was energy reminimized until the rms gradient became less than 0.01 kJ/mol. The low-energy structures of **1** and **2** were compared with that of U-turn structures as follows: First, the UpU structure having the same conformation as those of **1** and **2** was made by eliminating the $CH_2C(O)NHCH_2$ group and $C(O)NHCH_2$ group from **1** and **2**, respectively. The UpU structure having the same conformation as that of the U-turn of the $tRNA^{Phe}$ was constructed by replacing the G(34) residue of U(33)pG(34) dimer extracted from the X-ray crystallographic structure of $tRNA^{Phe}$ (obtained from PDB Brookhaven National Laboratory) with uracil. The UpU structures were superimposed with the aid of the method of the least-squares fit, and the rms (Å) values were calculated.

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Supporting Information Available: The ^{1}H, ^{13}C, and ^{31}P NMR spectra of compounds **1**−**29**, ^{1}H−^{1}H DQF COSY and NOESY spectra of compounds **1** and **2**, and HPLC profiles of the enzymatic digestion of compounds **1** and **2** (47 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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