Synthesis and Properties of Oligonucleotides Having a Phosphorus Chiral Center by Incorporation of Conformationally Rigid 5′-Cyclouridylic Acid Derivatives

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This paper describes the design and synthesis of a conformationally rigid dimer building block Umpc3Um as a chiral center at the phosphate group with the S/N junction where c3 refers to a propylene bridge linked between the uracil 5-position and 5′-phosphate group of pUm. The extensive H^1 NMR analysis of Umpc3Um suggests that the 5′-upstream Um has predominantly a C2′-endo conformation and the pc3Um moiety exists almost exclusively in a C3′-endo conformation. The absolute configuration of the diastereomers Umpc3Um(fast) (**8a**) and Umpc3Um(slow) (**8b**) was determined by CD spectroscopy as well as computer simulations. The oligonucleotides U_4[Umpc3Um-(fast)]U_4 (**13a**) and U_4[Umpc3Um(slow)]U_4 (**13b**) incorporating **8a** and **8b** were synthesized by use of the phosphoramidite building blocks **11a** and **11b**, respectively. The T_m experiments of the duplexes formed between these modified oligomers and the complementary oligomers imply that the modified oligomer **13a** having Umpc3Um(fast) has the *S*p configuration at the chiral phosphoryl group.

Introduction

We previously reported the synthesis of confomationally rigid 5′-cyclouridylic acid derivatives **1** and **2** as shown in Figure 1.[1−3] These studies revealed that the cyclouridylic acids **1** and **2** have the fixed torsion angles of α, *β*, and *γ*, defined in the backbone structure in RNA duplex, in a quite natural manner without strain.

Therefore, one can expect that, if compound **2** can be incorporated into RNA, two stereoisomers with the *R* and *S* configurations due to the phosphorus center will be generated. One of them has a typical α torsion angle, which corresponds to that of the A type-helical RNA strand, while the other has a different torsion angle with +120°. It is also expected that the ribose conformation of a nucleoside 5′-upstream from the cyclouridylic acid will be influenced in terms of the neutral phosphate group that serves as an electron-withdrawing effect. Now, we can expect that totally RNA oligonucleotides incorporating these *R* and *S* phosphorus centers would exhibit unique structures conformationally fixed. From the crystal structure of *Escherichia coli* $tRNA^{Phe}$,[4] we have also noticed many characteristic bent motifs with disorder structures of the base-orientation between proximal positions 7−8, 9−10, and 19−20 or in the regions of consecutive positions 16−18, 46−49, and 53−61. These unique structures are apparently essential for compact folding of the tRNA molecule. In other RNA loop regions, a variety of backbone structures have been found by several research groups.[5] In general, RNA holding occurs via change of torsion angles of the RNA backbone structure and/or the ribose puckering, especially via dramatic change of the torsion angle of the internucleotidic phosphate diester linkage.[4]

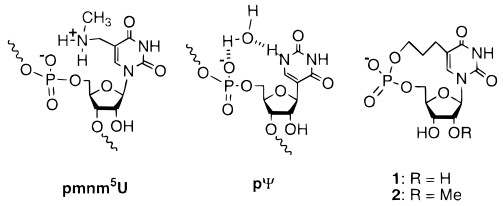

Figure 1. Intramolecular hydrogen bonds found in tRNAs and chemically synthesized covalent-bonding mimics **1** and **2**.

Therefore, it is interesting if various RNA structural motifs, formed between neighboring two ribonucleosides, can be artificially fixed by chemical methods. Such studies would provide a new insight in man-made functionalization of RNA as well as understanding of the structure−function relationship of RNA. To our best knowledge, no comprehensive studies have appeared

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(3) Sekine, M.; Kurasawa, O.; Seio, K.; Wada, T. *J. Org. Chem.* **2000**, *65*, 3571−3578.

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(5) The U-turn structure was found in hammerhead ribozymes and U2 snRNA: (a) Pley, H. W.; Flaherty, K. M.; McKay, D. B. *Nature* **1994**, *372*, 68−74. (b) Scott, W. G.; Finch, J. T.; Klug, A. *Nucl. Acids Symp. Ser.* **1995**, *34*, 214−216. (c) Scott, W. G.; Finch, J. T.; Klug, A. *Cell* **1995**, *81*, 991−1002. (d) Scott, W. G.; Murray, J. B.; Arnold, J. R. P.; Stoddard, B. L.; Klug, A. *Science* **1996**, *274*, 2065−2069.; (e) Stallings, S. C.; Moore, P. B. *Structure* **1997**, *5*, 1173−1185. In the loop of hepatitis delta virus antigenomic RNA a sharp reversed U-turn structure was found: (f) Kolk, M. H.; Heus, H. A.; Hilbers, C. W. *EMBO J.* **1997**, *16*, 3685−3692. The following papers reported the presence of the U-turn structure in a RNA hairpin loop: (g) Huang, S.; Wang, Y. X.; Draper, D. E. *J. Mol. Biol.* **1996**, *258*, 308−321. (h) Jucker, F. M.; Pardi, A. *RNA* **1995**, *1*, 219−222.

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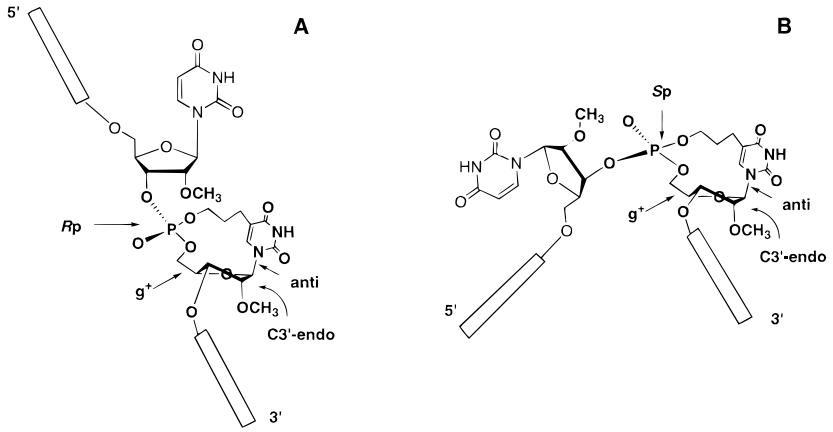

Figure 2. Schematic images of oligonucleotides incorporating a conformationally rigid cyclouridylic acid derivative pc3Um.

about the synthesis of oligonucleotides stereo-controlled simultaneously at the α, *β*, and *γ* positions to date.

In this paper, we report the synthesis of oligonucleotides incorporating a phosphorus chiral center by use of a conformationally fixed dimer building unit that has rigid sugar conformations, anti base-orientation, g^+ or t torsion angle around the P−$O^{5'}$ bond, and the typical A-type helical *β* and *γ* torsion angles.

Results and Discussion

RNA Bending Structures. Originally, 5′-uridylic acid derivatives **1**−**2** were designed as covalent-bonding mimics of conformationally rigid structural motifs, such as intramolecularly hydrogen-bonding 5-[(methylamino)-methyl]uridine 5′-phosphate (pmnm^{5}U)[6] and water-bridged pseudouridine 5′-phosphate (p*ψ*)[7] which are known to exhibit predominantly the C3′-endo conformation (A-type or N-type RNA) in the ribose moiety. We also disclosed that particularly the propylene-bridged 5′-cyclouridylic acids derivatives **1**[2] and **2**[3,8] have typical C3′-endo, anti, and g^+ conformations for the ribose moiety, glycoside bond, and C4′−C5′ torsion angle, respectively, which are actually the same as that of the A-type RNA strand.

This orientation, which produces a chiral phosphoryl center at the junction with the *R*p configuration, is the same as that of the usual A-type RNA strand. The other is depicted in the right side of Figure 2. This *S*p configuration can apparently create a bending structure if all the conformations of the cyclouridylic acid component can be maintained as in the original structure.

Our previous studies revealed that the fractional population of the C3′-endo conformation in **1** was 88%,[2a] which was estimated by the well-known equation of $P(\text{C3}'\text{-endo}) = J_{3',4'}/(J_{1',2'} + J_{3',4'})$.[9] Generally, the average value of the sum of $J_{1',2'}$ and $J_{3',4'}$ in many uridine derivatives is 9.4 Hz ± 0.2.[10] Deviation from this average value is a good indicator for estimation of the structural disorder. Particularly, the cyclouridylic acid **1** has proven to have a natural, favorable structure without strain, since this compound has actually a normal value (9.4 Hz) of the sum of $J_{1',2'}$ and $J_{3',4'}$.

On the basis of these promising conformational properties of **1**, we have recently reported that a decathymidylate pc3Um(pT)$_9$ incorporating the 2′-*O*-methylcyclouridylic acid derivative **2** (pc3Um) at the 5′-terminal end can form a more stable duplex with A(pA)$_9$ than the unmodified decathymidylate T(pT)$_9$.[3] This study also revealed that introduction of a 2′-*O*-methyl group into **1** can enhance the fractional population of the C3′-endo conformation up to more than 89%.

Synthesis of Umpc3Um (8) Involving the 5′-Cyclouridylic Acid Derivative pc3Um (2). To incorporate a dimer motif having a phosphorus chiral center into RNA oligomers, we synthesized Umpc3Um (**8**), which contains a cyclic structure at the 3′-downstream site. The 2′-hydroxy group of the 3′-downstream uridine was in advance methylated to circumvent the difficult selective 2′-*O*-protection. It is known that if the 2′-hydroxy group is free and a dialkoxyphosphoryl [−P(O)(OR)$_2$] group is attached to the neighboring 3′-oxygen, 3′ → 2′ phosphoryl migration inevitably occurs with cyclization of the phosphoryl group and elimination of an alcohol.[11] Accordingly, the 2′-hydroxy group of the 5′-upstream uridine was also masked with a methyl group to avoid such side reactions associated with the pc3Um residue.

We adopted a straightforward method for the simultaneous phosphotriester bond formation between a nucleoside and a nucleotide component (Scheme 1): The 2′-*O*-methyluridine 3′-phosphorodiamidite derivative **4** was prepared by reaction of 5′-*O*-DMTr-2′-*O*-methyluridine (**3**) with bis(diisopropylamino)chlorophosphine according to the method reported by van Boom et al.[12] Condensation of **4** with the 2′-*O*-methyluridine diol derivative **5**[3] gave

(6) (a) Sakamoto, K.; Kawai, G.; Niimi, T.; Watanabe, S.; Niimi, T.; Satoh, T.; Sekine, M.; Yamauti, Z.; Nisimura, S.; Miyazawa, T.; Yokoyama, S. *Eur. J. Biochem*. **1993**, *216*, 369−375. (b) Sakamoto, K.; Kawai, G.; Watanabe, S.; Niimi, T.; Hayasi, N.; Muto, Y.; Watanabe, K.; Satoh, T.; Sekine, M.; Yokoyama, S. *Biochemistry* **1996**, *35*, 6533−6538.

(7) Davis, D. R.; Poulter, C. D. *Biochemistry* **1991**, *30*, 4223−4231.

(8) In the case of **2** having the 2′-*O*-methyl group, the resonance signal of 1′-H was observed as a singlet, suggesting the $J_{1',2'}$ coupling constant is less than 1.0 Hz. Judging from the $J_{3',4'}$ value (8.1 Hz) of **2**, the fractional population of its C3′-endo conformation was expected to be at least more than 89%.[3] In this simulation, the sum of $J_{1',2'}$ and $J_{3',4'}$ of **2** is estimated to be less than 9.1 Hz, which is relatively close to the standard value of 9.4 ± 0.2 Hz.[10] Therefore, the sugar puckering of **2** is faintly disordered because of the presence of both the cyclic structure and the 2′-*O*-methyl group but is essentially similar to that of **1**.

(9) Altona, C.; Sundaralingham, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333−2344.

(10) Davies, D. B. *Prog. Nucl. Magn. Reson. Spectrosc.* **1978**, *12*. 135−225.

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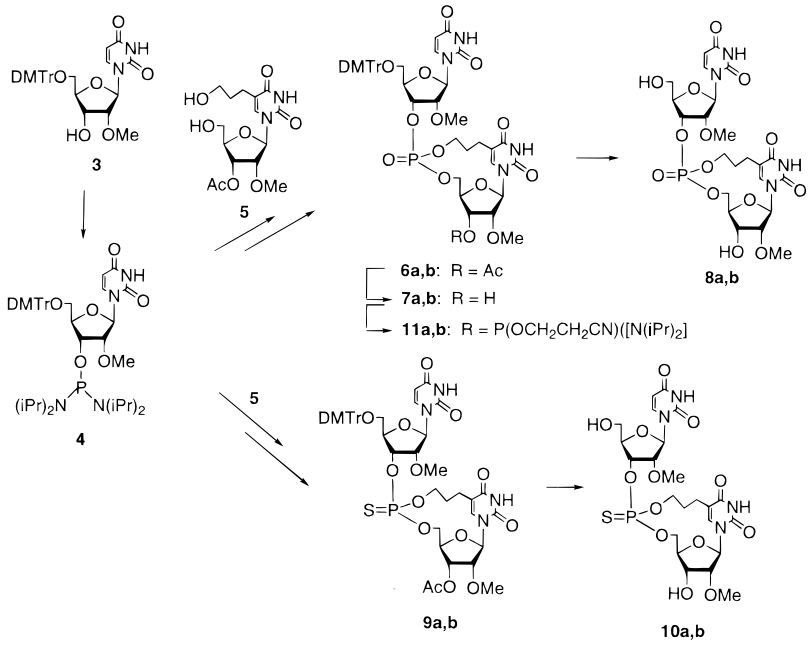
(12) Marugg, J. E.; Burik, A.; Tromp, M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2271−2274.

Scheme 1



the coupling product **6** as a diastereomeric mixture in 80% yield. It should be noted that this internucleotidic phosphotriester bond formation proceeded effectively without using any techniques such as the high dilution method often used in the synthesis of macrocyclic compounds.[13] In other words, this result implies that the 12-membered ring cyclic structure fits reasonably the naturally occurring cyclic hydrogen bonding systems.

Deacetylation of **6** gave a pair of diastereomeric products DMTrUmpc3Um (**7a,b**) without damage of the phosphotriester linkage. Fortunately, the stereoisomers **7a** (fast-eluted product) and **7b** (slow-eluted product) could be readily separated by silica gel column chromatography and successfully isolated in 46 and 43% yields, respectively. Treatment of each diastereomer **7a** or **7b** with 80% acetic acid gave the corresponding product Umpc3Um(fast) (**8a**) or Umpc3Um(slow) (**8b**).

Conformational Analysis of Umpc3Um (8a,b) Involving a Cyclic Phosphotriester Structure. First, it should be taken into account that the neutral phosphoryl moiety in **8a,b** affects conformation of the original ribose moieties of the Um and pc3Um components. Generally, the dialkoxyphosphoryloxy [−OP(O)(OR)$_2$] group serves as a strong electron-withdrawing group, while the monoalkoxyphosphoryloxy [−OP(O)(OR)(O$^-$)] group behaves as a weakly electron-withdrawing group.[14−16] Basically, the electronegativity of a substituent attached to the 2′ or 3′-carbon is a key factor for control of the ribose pucker, as reported by Ikehara.[17] The 3′-oxygen of the neutral phosphoryl group increases its electronegativity due to the electron-withdrawing effect of the [−P(O)(OR)$_2$] moiety so that the ribose conformation of the 5′-upstream Um can be expected to be strongly affected. Therefore, we examined if this difference leads to significant conformational change of both the 3′-downstream cyclonucleoside and the 5′-upstream nucleoside in the modified dimer **8a,b**.

The 1′-H proton of the 3′-downstream pc3Um in **8a** was observed as a doublet with $J_{1',2'}$ value of 1.7 Hz, which shows 82% of C3′-endo conformation on the assumption that the $J_{1',2'} + J_{3',4'}$ value is equal to that of pU (9.2 Hz), as shown in Table 1. Furthermore, it is noteworthy that the 1′-H proton of the 3′-downstream pc3Um of **8b** appeared as a singlet within the resolution of the NMR apparatus. This means that the sugar pucker of the 3′-downstream pc3Um has an extremely predominant C3′-endo conformation. From these results, it turned out that the phosphotriester moiety did not affect the whole conformation of the 3′-downstream pc3Um of **8a** and **8b**.

Expectedly, the sugar puckers of the 5′-upstream Um in **8a** and **8b** were maintained predominantly in the C2′-endo (B-type) conformation to the extent of 70% and 73%, respectively, regardless of the presence of the 2′-*O*-methoxy group, which is known to induce the C3′-endo conformation.[18]

These results suggest that the dimers **8a** and **8b** have conformationally locked C3′-endo and C2′-endo sugar puckers at the 3′-downstream and 5′-upstream components, respectively. In yeast tRNA^{Phe} there exists a sharply bent dimer motif with a sequence of U(7)pU(8) having the C2′-endo and C3′-endo conformations at positions 7 and 8, respectively.[19] This fact is interesting since our dimer model of either **8a** or **8b** could be used as a conformationally restrained mimic of such a sharply bent motif. Therefore, we needed to determine which

(13) For a recent paper see Sinha, S. C.; Keinan, E. *J. Org. Chem.* **1997**, *62*, 377−386 and cited papers therein.

(14) Hansch, C.; Leo, A.; Taft, W. *Chem. Rev.* **1991**, *91*, 165−195. For example, the diethoxyphosphoryl group exhibits a field/inductive effect having the *F* value of 0.52,[15] while the *F* values of the acetyl and acetoxy groups are 0.33 and 0.42, respectively.[17] Since the *F* value of the dissociated phosphoryl group PO_3H^- is 0.19,[17] monoalkoxyphosphoryl P(O)(OR)O$^-$ group should have a little lower *F* value than 0.19, which means a weakly electron-withdrawing group.

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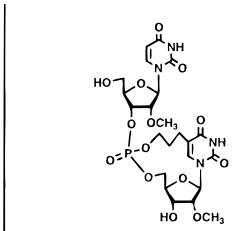
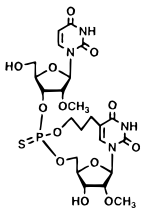
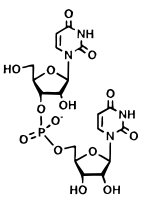
(17) Ikehara, M. *Heterocycles* **1984**, *21*, 75−90.

(18) Kawai, G.; Yamamoto, Y.; Kamimura, T.; Masegi, T.; Sekine, M.; Hata, T.; Iimori, T.; Watanabe, T.; Miyazawa, T.; Yokoyama, S. *Biochemistry* **1992**, *31*, 1040−1046.

(19) Protein Databank, Protein Institute of Osaka University, Japan.

(20) Davies, D. B.; Danyluk, S. S. *Biochemistry* **1974**, *13*, 4417-4434.

Table 1. Fractional Populations of C3′-Endo Conformation of the 5′-Upstream and 3′-Downstream Nucleosides of Dimers Containing a Cyclouridylic Acid Derivative and Related Compounds

	Umpc3Um(fast) 8a		Umpc3Um(slow) 8b		Umpsc3Um(fast) 10a		Umpsc3Um(slow) 10b		UpU	
J (Hz)	Ump	pc3Um	Ump	pc3Um	Ump	psc3Um	Ump	psc3Um	Up-[a]	-pU[a]
$J_{1,2'}$	6.6	1.7	6.6	singlet	6.9	singlet	6.9	1.6	4.2	3.9
$J_{1,2''}$	—	—	—	—	—	—	—	—	—	—
$J_{2',3'}$	4.9	5.0	4.8	4.9	4.9	4.5	4.9	5.0	5.2	5.1
$J_{2'',3'}$	—	—	—	—	—	—	—	—	—	—
$J_{3',4'}$	2.8	—	2.5	—	2.3	—	2.8	7.4	5.3	5.0
$J_{1,2'}+J_{3',4'}$	9.4	—	9.1	—	9.2	—	9.7	9.0	9.5	8.9
P(C3'-endo)[c]	30%	82%	27%	~90%	25%	~90%	29%	82%	56% 53%[b]	56% 63%[b]

[a] Reference 20. [b] Reference 18. [c] The fractional populations of ribose residues were calculated according to ref 9.

stereoisomer **8a** or **8b** has a bent structure. This determination was done as described in a later section.

Electronic Effect of a 5′-Upstream 3′-Thiophosphoryl Substituent on Conformation of the Um Ribose. To check the electronic effect of the phosphoryl group on the conformation of the 5′-upstream Um, the cyclic phosphorothioate derivatives Umspc3Um (**10a,b**) were synthesized via the intermediate **9**, which could be obtained by sulfurization of the common intermediate obtained by condensation of **4** with **5**. The cyclic phosphorothioates **10a** (fast-eluted product) and **10b** (slow-eluted product) were separated and isolated. The ^{1}H NMR analysis of these stereoisomers showed essentially similar results but a singlet signal for the 1′-H proton of the 3′-downstream pc3Um in the fast-eluted isomer **10a** was observed, and the slow-eluted isomer **10b** has the $J_{1',2'}$ value of 1.6 Hz (see Table 1).

These results suggest that the thiophosphoryl group did not afford the conformational change of the 5′-upstream Um appreciably, although the sulfur atom is less electronegative than the oxygen.

Computational Analysis of 3D-Structures of Umpc3Um 8 Capable of Duplex Formation with RNA and DNA. The structures of the *R*p and *S*p stereoisomers of Umpc3Um were examined by computational methods. As mentioned above, the 5′-upstream Um ribose moieties of **8a** and **8b** predominantly exist in the C3′-endo conformation while the 3′-downstream pc3Um has the C3′-endo conformation. Accordingly, an initial 3D model was constructed by using the B-type uridine 3′-phosphate and the A-type uridine structures extracted from the canonical B-type RNA and A-type RNA duplexes, respectively. The initial *R*p and *S*p model structures of Umpc3Um **8** were built by using these fragments and adding the methyl groups, and many conformers were generated according to the Monte Carlo method of MacroModel ver 5.0.[21] Each conformer was energy-minimized with the implicit solvent model, GB/SA.[22] This calculation was carried out by constraining the 3′-downstream c3Um in the C3′-endo conformation, which has proven to be apparently predominant from the NMR analysis. The 5′-upstream Um was also constrained as the C2′-endo (major conformation) or C3′-endo form (minor conformation). The results of these calculations are shown in Figure 3A−D. At the upper left side of Figure 3, UpU extracted from the typical A-type RNA duplex is also shown.

In the case of the *R*p isomer having only the 3′-endo conformation in both the 5′-upstream Um and 3′-downstream pc3Um, the most stable conformation (Figure 3A) was similar to that of UpU extracted from the A-type RNA duplex. Therefore, it can be expected that the dimer with this *R*p configuration and the C3′-endo/C3′-endo conformations can hybridize with ApA in a natural way. In contrast, the most stable structure (Figure 3B) of the *S*p stereoisomer has a conformation entirely different from that of UpU extracted from the A-type RNA duplex. This conformer forms a normal duplex in a straight direction only with difficulty.

On the other hand, the calculated structure (Figure 3C) of the *R*p stereoisomer having the C2′-endo and C3′-endo conformations for the 5′-upstream Um and the 3′-downstream pc3Um, respectively, showed the stacked structure of the two uracil rings with different orientations with each other, suggesting that formation of the duplex with ApA is impossible. The *S*p isomer (Figure 3D) having the C2′-endo and C3′-endo conformations for the 5′-upstream Um and the 3′-downstream pc3Um, respectively, has a conformation considerably different

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(22) Still, W. C.; Tempczyk, A.; Hawley, R. C.; Hendrickson, T. *J. Am. Chem. Soc.* **1990**, *112*, 6127−6129.

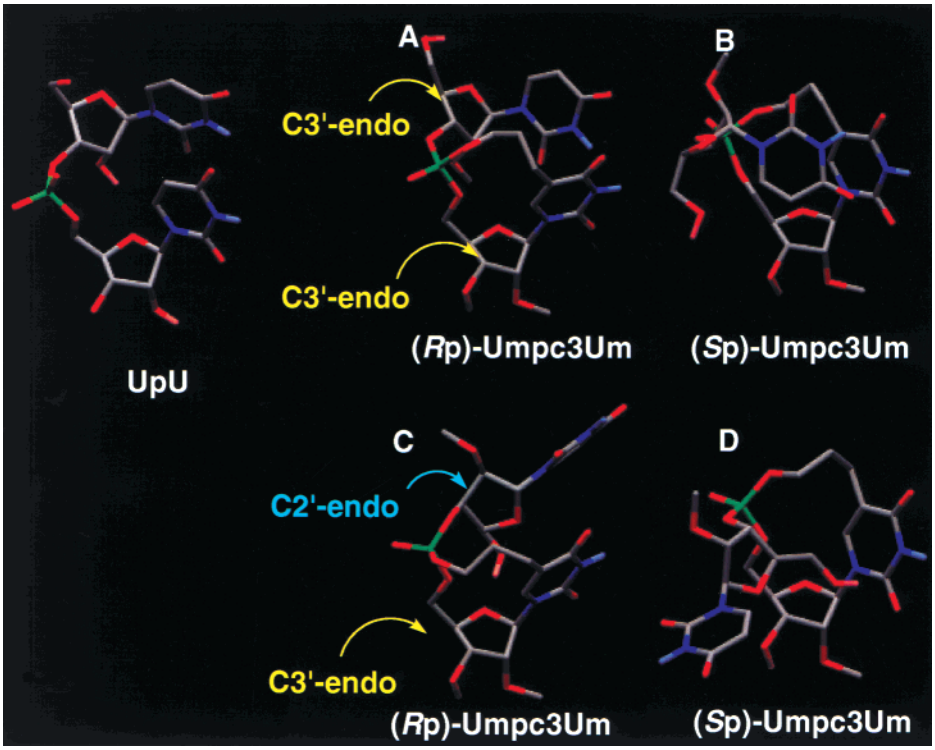


Figure 3. Energy-minimized structures of (*R*p)-Umpc3Um and (*S*p)-Umpc3Um in which the 5′-upstream ribose residue was fixed in either C3′-endo or C2′-endo conformation and the 3′-downstream ribose residue was fixed in the C3′-endo conformation. (A) (*R*p)-Umpc3Um with C3′-endo/C3′-endo; (B) (*S*p)-Umpc3Um with C3′-endo/C3′-endo; (C) (*R*p)-Umpc3Um with C2′-endo/C3′-endo; (D) (*S*p)-Umpc3Um with C2′-endo/C3′-endo.

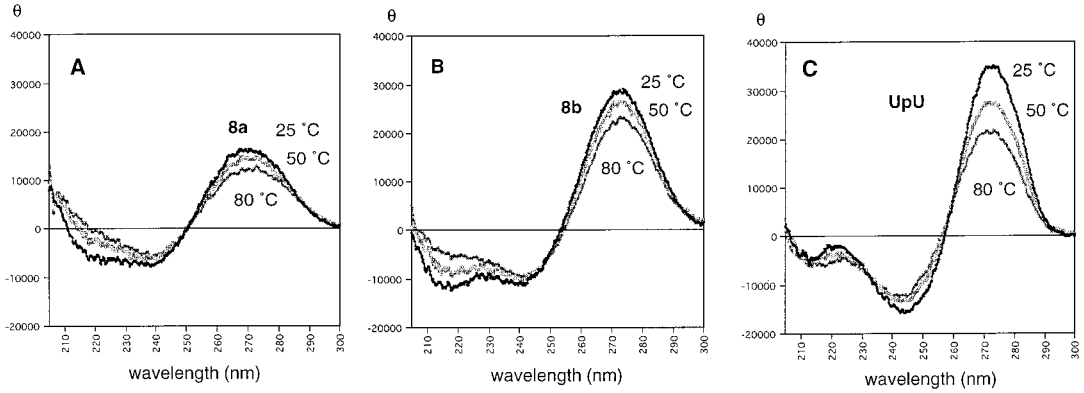


Figure 4. CD spectra of Umpc3Um(fast) (**8a**) (A), Umpc3Um(slow) (**8b**) (B), and UpU (C) in phosphate buffer (1.0 A260/1 mL, pH 7.0, 25, 50, 80 °C)

from that of UpU so that this isomer cannot form a stable duplex with ApA.

These calculations suggest that the *R*p isomer of Umpc3Um can form a normal duplex only when the 5′-upstream Um takes the minor C2′-endo conformation, but the *S*p isomer cannot give such a normal linear duplex in any cases. Therefore, it was expected that duplexes formed between oligonucleotides containing *R*p-Umpc3Um and their complementary oligonucleotides should have higher T_m values than those formed between oligonucleotides containing *S*p-Umpc3Um and their complementary oligonucleotides. This prediction can be used for determination of the absolute configuration of **8a** and **8b**.

CD Measurements of 8a and 8b. To determine the absolute configuration of the fast-eluted Umpc3Um **8a** and the slowly eluted Umpc3Um **8b**, the CD spectra of these compounds were measured in phosphate buffer (pH 7.0) at 25, 50, and 80 °C. These results are shown in Figure 4A,B. It is clearly shown that the intensity of the positive Cotton effect of **8b** around 260 nm is ca. twice that of **8a**. The negative Cotton effects around 240 and 215 nm can be seen at 25 °C in **8b**, but these features typical for UpU cannot be clearly observed in **8a**. These results strongly suggested that the slow-eluted isomer **8b** has the *R*p configuration, which allows us to give a structure closer to that of UpU, as suggested by the above computer simulation.

Incorporation of Cyclouridylic Acid Derivatives into Decauridylate and Decathymidylate. To incorporate a conformationally rigid C2′-endo/C3′-endo (i.e., S/N) junction unit Umpc3Um into RNA and DNA oligomers, the phosphoramidite units **11a** and **11b** were synthesized in high yields from **7a** and **7b**, respectively. The decauridylate derivatives U_4[Umpc3Um(fast)]U_4 (**13a**) and U_4[Umpc3Um(slow)]U_4 (**13b**) incorporating the S/N

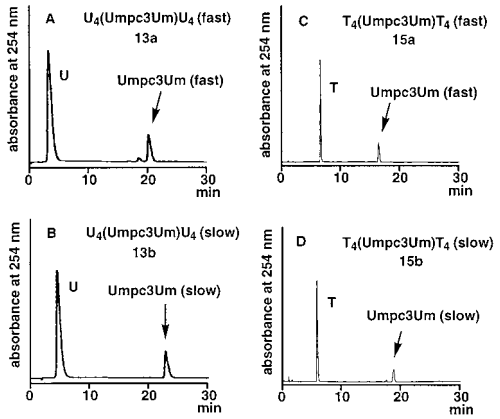
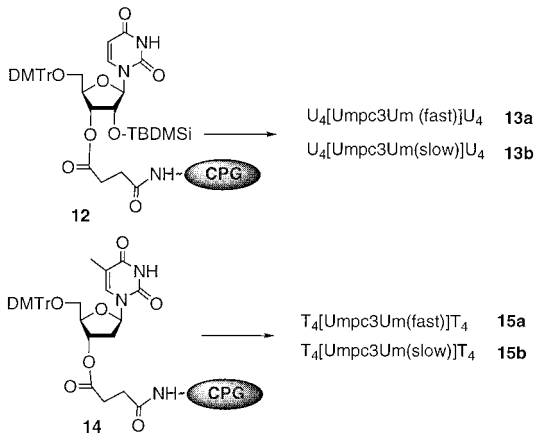


Figure 5. Enzymatic digestion of the decauridylates **13a** and **13b** and decathymidylates **15a** and **15b** incorporating a cyclouridylic acid unit of Umpc3Um(fast) or Umpc3Um(slow) with venom phosphodiesterase and alkaline phosphatase. The differences of Umpc3Um in retention time between A, B and C, D arose from different HPLC apparatus used.

Scheme 2



junction units **8a** and **8b** were obtained in 22% and 18% yields, respectively, by using the standard solid support synthesis starting from an U-loaded CPG gel **12**. Similarly, decathymidylate derivatives T_4[Umpc3Um(fast)]T_4 (**15a**) and T_4[Umpc3Um(slow)]T_4 (**15b**) incorporating the S/N junction units **8a** and **8b** were obtained in 58% and 25% yields, respectively, from a T-loaded CPG gel **14**. See Scheme 2.

Enzymatic Analysis of Oligonucleotides Incorporating a Cyclouridylic Acid Derivative. The cyclouridylic acid derivatives **1** and **2** were found to be completely resistant to venom phosphodiesterase, spleen phosphodiesterase, and nuclease P1 under the standard conditions. Therefore, the cyclic bridge structure inhibits the enzyme activity. This inhibition property can be used for characterization of the structure of oligonucleotides incorporating a cyclic structure. When U_4[Umpc3Um(fast)]U_4 (**13a**) and U_4[Umpc3Um(slow)]U_4 (**13b**) were incubated with venom phosphodiesterase followed by alkaline phosphatase, the cyclic dimer fragments of Umpc3Um(fast) (**8a**) and Umpc3Um(slow) (**8b**) were observed with the concomitant formation of uridine in the correct ratios, as shown in Figure 5A,B, respectively. Similarly, the enzyme digestion of T_4[Umpc3Um(fast)]-T_4 (**15a**) and T_4[Umpc3Um(slow)]T_4 (**15b**) gave the same dimer fragments of Umpc3Um(fast) (**8a**) and Umpc3Um-(slow) (**8b**), as shown in Figure 5C,D, respectively.

Table 2. T_m Values of the Duplexes between Modified Oligonucleotides and Decaadenylate or Decadeoxyadenylate

	melting temp of duplex (°C)			
oligonucleotides	$A(pA)_9$	ΔT_m	d[$A(pA)_9$]	ΔT_m
$U(pU)_9$	16.7		13.3	
U_4[Umpc3Um(fast)]U_4 (**13a**)	<0	>−16.7	not obsd	
U_4[Umpc3Um(slow)]U_4 (**13b**)	6.8	−9.9	6.3	−7.0
$T(pT)_9$	20.3		27.0	
T_4[Umpc3Um(fast)]T_4 (**15a**)	8.2	−12.1	<5	>−22.0
T_4[Umpc3Um(slow)]T_4 (**15b**)	16.3	−4.0	17.0	−10.0

Table 3. Synthesis of Dinucleotides Containing a Cyclouridylic Acid Unit

protected dimer	yield, %	dimer	HPLC ret time,[b] min	yield, %	isolated yield by HPLC, %
6a,b	80				
7a	46	**8a**	20.8	82	
7b	43	**8b**	23.2	79	
9a,b	43	**10a**	17.3	91	21
		10b	19.3		22

[a] TLC was carried out by using the following solvent system: $CHCl_3$−Et_2O−MeOH (20:10:3, v/v/v, three times). [b] HPLC was performed under the conditions described in the experimental section.

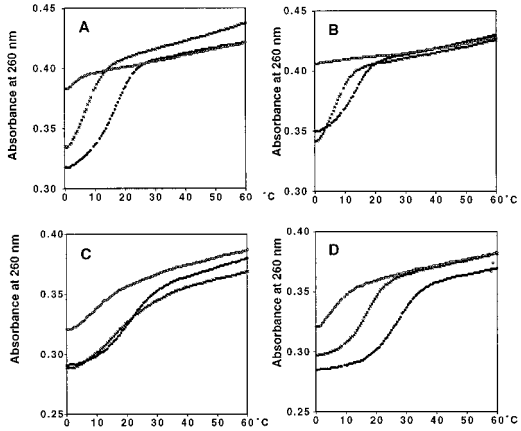


Figure 6. Melting curves of the duplexes formed between $A(pA)_9$ or d[$A(pA)_9$] and oligonucleotides **13** or **15**. Panel A: (○) $A(pA)_9$−U_4[Umpc3Um(fast)]U_4(**13a**), (×) $A(pA)_9$−U_4-[Umpc3Um(slow)]U_4(**13b**), (●) $A(pA)_9$−$U(pU)_9$. Panel B: (○) d[$A(pA)_9$]−U_4[Umpc3Um(fast)]U_4(**13a**), (×) d[$A(pA)_9$]−U_4-[Umpc3Um(slow)]U_4(**13b**), d[$A(pA)_9$]−$U(pU)_9$. Panel C: (○) $A(pA)_9$−T_4[Umpc3Um(fast)]T_4(**15a**), (×) $A(pA)_9$−T_4[Umpc3Um-(slow)]T_4U_4(**15b**), (●) $A(pA)_9$−$T(pT)_9$. Panel D: (○) d[$A(pA)_9$]−T_4[Umpc3Um(fast)]T_4(**15a**), (×) d[$A(pA)_9$]−T_4[Umpc3Um(slow)]-T_4(**15b**), (●) d[$A(pA)_9$]-$T(pT)_9$.

Thermal Stability of Duplexes of U_4[Umpc3Um-(fast)]U_4 (13a) and U_4[Umpc3Um(slow)]U_4 (13b) with $A(pA)_9$ and d[$A(pA)_9$]. We studied the thermal stabilities of all possible four duplexes of U_4[Umpc3Um-(fast)]U_4 (**13a**) and U_4[Umpc3Um(slow)]U_4 (**13b**) with the complementary oligonucleotides $A(pA)_9$ and d[$A(pA)_9$] compared with those of the reference duplexes $A(pA)_9$−$U(pU)_9$ and d[$A(pA)_9$]−$U(pU)_9$. It is also of great interest to examine how the duplexes are affected by the presence of the S/N junction point. The results of the T_m experiments of all the possible duplexes are summarized in Table 2 and Figure 6A,B. The duplex of $A(pA)_9$−U_4-[Umpc3Um(fast)]U_4(**13a**) shows a little change in the melting curve, as shown in Figure 6A. On the other hand, the melting curve of $A(pA)_9$−U_4[Umpc3Um(slow)]U_4(**13b**) shows a more typical sigmoid curve, giving a higher T_m

of 6.8 °C, which is considerably low compared with that of $A(pA)_9-U(pU)_9$.

The duplex of $d[A(pA)_9]-U_4$[Umpc3Um(fast)]U_4(**13a**) shows no clear T_m curve, as shown in Figure 6B. In contrast, the duplex of $d[A(pA)_9]-U_4$[Umpc3Um(slow)]-U_4(**13b**) shows a sigmoid curve with a lower T_m value (7.0 °C) than that of $d[A(pA)_9]-U(pU)_9$.

In these T_m experiments, the decauridylate **13b** containing Umpc3Um(slow) (**8b)** shows unexceptionally higher T_m values than **13a** containing Umpc3Um(fast) (**8a**). Therefore, these results indicate that Umpc3Um-(slow) **8b** has the *R*p configuration and Umpc3Um(fast) **8a** has the *S*p configuration, which does not allow favorable duplex formation. This conclusion is in agreement with that from the CD analysis.

Thermal Stability of Duplexes of T_4[Umpc3Um-(fast)]T_4 15a and T_4[Umpc3Um(slow)]T_4 15b with $A(pA)_9$ and $d[A(pA)_9]$. Figure 6C shows the melting curves of three kinds of duplexes, i.e., $A(pA)_9-T_4$[Umpc3Um(fast)]T_4(**15a**), $A(pA)_9-T_4$[Umpc3Um(slow)]-T_4(**15b**), and $A(pA)_9-T(pT)_9$. As seen in Figure 6C, there is a big difference in T_m between $A(pA)_9-T_4$[Umpc3Um-(fast)]T_4(**15a**) and $A(pA)_9-T_4$[Umpc3Um(slow)]T_4(**15b**). The latter has the T_m value of 16.3 °C, which is lower by 4.0 °C than that of $A(pA)_9-T(pT)_9$, while the former showed a very low T_m value of 8.2 °C. Figure 6D shows the melting curves of the duplexes of $d[A(pA)_9]-T_4$[Umpc3Um(fast)]T_4(**15a**), $d[A(pA)_9]-T_4$[Umpc3Um-(slow)]T_4(**15b**), and $d[A(pA)_9]-T(pT)_9$. In the case of $d[A(pA)_9]-T_4$[Umpc3Um(slow)]T_4(**15b**), the T_m value was considerably low (17.0 °C) compared with that (27 °C) of $d[A(pA)_9]-T(pT)_9$. Furthermore, the duplex of $d[A(pA)_9]-T_4$[Umpc3Um(fast)]T_4(**15a**) has a much lower T_m value (5.8 °C). This marked decrease (21.2 °C) is probably one of the extreme cases reported so far. These results also support our previous conclusion that Umpc3Um(fast) (**8a**) should have the *S*p configuration.

Conclusions

In this study, it was found that the 5′-upstream 2′-*O*-methyluridine having a cyclic structure has the C2′-endo conformation predominantly over the C3′-endo conformation. On the other hand, the 3′-downstream 2′-*O*-cyclouridine of the dimer exists in favor of the C3′-endo conformation because of the rigid structure. All duplexes between decathymidylates or decauridylates incorporating Umpc3Um(fast or slow) and the complementary decadeoxyadenylates or decaadenylates showed decrease of the T_m values. Particularly, decanucleotides having Umpc3Um(fast) exhibited significantly lower T_m values than those having Umpc3Um(slow).

Molecular mechanics calculations of two stereoisomers of Umpc3Um indicate that the *R*p isomer can form relatively normal duplexes when the 5′-upstream uridine turns back to the C3′-endo conformation but the *S*p isomer is unfavorable for stable duplex formation even in any combination of the C2′-endo and C3′-endo conformations in the 5′-upstream and 3′-downstream uridine moieties. From these results, it could be concluded that Umpc3Um(fast) is the *S*p isomer and Umpc3Um(slow) is the *R*p isomer. Although the 5′-upstream nucleoside residue tends to have the C2′-endo conformation, decanucleotides having (*R*p)-Umpc3Um are capable of duplex formation with a disordered partial structure. In addition, it is also suggested that in Umpc3Um (*S*p) the orientation of the phosphate moiety is unfavorable for duplex formation. In other words, there is a possibility that the backbone structure can be artificially bent by use of this unfavorable stereoisomer as the bending point.

Finally, it should be noted that such an S/N junction actually exists in naturally occurring RNA molecules. After extensive search, we noticed that in the crystal structure of *E. coli* $tRNA^{Phe}$ this S/N junction of U(7)pU-(8) sequence is present and plays a role in changing the orientation of the base vectors at the position between the acceptor- and D-stems.[4a] Therefore, our structural motif having a S/N junction can also be used to fix the bending in certain RNAs.

It is also interesting to introduce a rigid C3′-endo nucleoside into the 5′-upstream nucleoside of the UpU dimer motif. As such a rigid nucleoside, bicyclonucleoside derivatives recently reported by Imanishi[23k,l] and Wengel[23m,n] are facinating, since the sugar conformation of these locked nucleosides must be unaffected by the presence of a 3′-phosphotriester moiety. Further studies are now under investigation.

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 $^1H-^1H$ coupling constants were measured at 400 MHz using Varian Unity 400 spectrometer at 20 °C. UV spectra were recorded on a U-2000 spectrometer. CD spectra were recorded on J-500 C spectrometer using a 0.5 cm cell. The synthesis of oligonucleotides was performed on a DNA synthesizer model 381A or DNA/RNA synthesizer model 392. TLC was performed by the use of Kieselgel 60-F-254 (0.25 mm). Column chromatography was performed with silica gel C-200 purchased from Wako Co. Ltd., 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 using the following systems. System 1: An LC module 1 was used with an M-741 data module and a μBondasphere 5 μ C18

(23) In connection with the present study, there have been reported conformationally locked nucleosides: (a) Tarköy, M.; Bolli, M.; Schweizer, B.: Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481−510. (b) Egli, M.; Lubini, P.; Bolli, M.; Dobler, M.; Leumann, C. *J. Am. Chem. Soc.* **1993**, *115*, 5855−5856. (c) Tarköy, M.; Leumann, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1432−1434. (d) Tarköy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta* **1994**, *77*, 716−744. (e) Bolli, M.; Lubini, P.; Leumann, C. *Helv. Chim. Acta* **1995**, *78*, 2077−2096. (f) Litten, C. J.; Epple, C.; Leumann, C. J.; *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1231−1234. (g) Litten, J. C.; Leumann, C. *Helv. Chim. Acta* **1996**, *79*, 1129−1146. (h) Jones, R. J.; Swaminathan, S.; Milligan, J. F.; Wadwani, S.; Froehler, B. C.; Matteucci, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 9816−9817. (i) Zou, R.; Matteucci, M. D. *Tetrahedron Lett.* **1996**, *37*, 941−944. (j) Altmann, K.-H.; Kesselring, R.; Francotte, E.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 2331−2334. (k) Altmann, K.-H.; Imwinkelried, R.; Kesselring, R.; Rihs, G. *Tetrahedron Lett.* **1994**, *35*, 7625−7628. (l) Herdewijn, P. *Liebigs Ann.* **1996**, 1337−1348. (m) Egli, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1894−1909. (n) Ezzitouni, A.; Russ, P.; Marquez, V. E. *J. Org. Chem.* **1997**, *62*, 4870−4873. (o) Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735−8738. (p) Obika, S.; Morio, K.; Nanbu, D.; Imanishi, T. *Chem. Commun.* **1997**, 1643−1644. (q) Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401−5404. (r) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455−456. (s) Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607−3630. (t) Christensen, N. K.; Petersen, M.; Nielsen, P.; Jacobsen, J. P.; Olsen, C. E.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 5458−5463. (u) Wang, G.; Gunic, E.; Girardet, J.-L.; Stoisavljevic, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1147−1150.

100 Å column (3.9 × 150 mm) at 50 °C with a linear gradient (0−30%) of CH_3CN in 0.1 M NH_4OAc, pH 7.0 at a flow rate of 1.0 mL/min for 30 min. System 1b (for **10a,b**): The same conditions as described in system 1 were used except for the conditions of a linear gradient (0−50%) of CH_3CN in 0.1 M NH_4OAc, pH 7.0 for 25 min. System 2: A 2690 separation module was used with a 996 photodiode array detector and a Millennium 2010 chromatography manager. The other conditions are the same as those of system 1. System 3: A Shimazu SCL-6A was used with LC-6A, SPD-6A, and C-R3A. The other conditions are the same as those of system 1 except for a flow rate of 3.0 mL/min. Anion-exchange HPLC was done on an LC module 1 with an M-741 data module and a column heater with a 10−67% linear gradient of 25 mM phosphate, 1 M sodium chloride buffer (pH 6.9) in 25 mM phosphate buffer (pH 6.0) at a flow rate of 1.0 mL/min for 40 min. Pyridine was distilled two times from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves 4 Å. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-methyluridine 3′-(*N,N,N′,N′*-tetraisopropyl)phosphorodiamidite (4). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-methyluridine (1.12 g, 2.0 mmol) was coevaporated three times with dry toluene and finally dissolved in dry dioxane (10 mL). To this solution were added triethylamine (834 ul, 6.0 mmol) and bis(diisopropylamino)-chlorophosphine[26] (1.05 g, 4.0 mmol). The resulting mixture was stirred at room temperature for 2 h and then quenched by addition of ethanol (1 mL). The mixture was diluted with $CHCl_3$ (50 mL). The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (2 mL), and this solution was added portionwise to hexane (100 mL) with vigorous stirring. The resulting white precipitate was collected by removal of the solvent by decantation and dried in vacuo to give **4** (930 mg, 59%): ^{31}P NMR (109 MHz, $CDCl_3$) δ 119.12. This compound was used for the next experiment without further purification because of its extreme instability.

Synthesis of Fully Protected 2′-*O*-Methyl-2′-deoxyuridylyl(3′-5′)2′-*O*-methyldeoxyuridine Derivative 6a,b Having a Cyclic Structure with a Propylene Bridge. A mixture of **5** (358 mg, 1.0 mmol) and 1*H*-tetrazole (420 mg, 6.0 mmol) was rendered anhydrous by coevaporation with dry toluene and the residue was dissolved in dry acetonitrile (50 mL) and dioxane (50 mL). A solution of **4** (1.0 mmol) in acetonitrile (2.0 mL) was added dropwise to the mixture over a period of 5 min. The resulting mixture was stirred at room temperature for 1 h and a solution of **4** (0.50 mmol) in acetonitrile (1.0 mL) was added. After stirring was continued for an additional 4 h, *t*-BuOOH (1.0 mL, 10.0 mmol) was added to the mixture. The solution was evaporated under reduced pressure after stirring for 30 min, and the residue was extracted with $CHCl_3$ (100 mL) and 5% $NaHCO_3$ (100 mL). The organic layer was collected, washed two times with 5% $NaHCO_3$ (10 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (50 g) with $CHCl_3$−MeOH (100:2, v/v) containing 1% pyridine to give DMTrUmpc3Um(OAc) **6a,b** as foam (771 mg, 80%, the ratio of the diasteromers 1:1): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.64−2.04 (4H, m), 2.14 (6H, s), 2.35−2.55 (4H, m), 3.37−4.43 (37H, m), 4.67−4.72 (1H, m), 4.85−4.96 (2H, m), 5.00−5.18 (2H, m), 5.21−5.29 (2H, m), 5.99−6.04 (4H, m), 6.84−6.87 (8H, m), 7.15−7.34 (18H, m), 7.73 (2H, s), 7.91−7.95 (2H, m), 9.78−9.90 (4H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 20.56, 20.81, 21.46, 27.01, 55.27, 58.74, 58.94, 60.65, 63.22, 64.10, 66.04, 68.68, 69.06, 72.74, 73.69, 79.53, 81.01, 81.15, 81.76, 81.85, 82.30, 82.68, 87.17, 87.40, 88.03, 88.21, 102.60, 110.58, 110.87, 113.35, 127.47, 128.10, 128.23, 128.34, 129.04, 130.28, 134.50, 134.54, 134.70, 137.28, 139.51, 139.60, 143.68, 143.75, 149.67, 150.11, 150.17, 150.33, 150.38, 158.87, 163.36, 163.52, 163.57, 170.01, 170.22; ^{31}P NMR (109 MHz, $CDCl_3$) δ −2.32, −1.88. Anal. Calcd for $C_{46}H_{51}N_4O_{17}P$: C, 57.38; H, 5.34; N, 5.82. Found: C, 57.00; H, 5.58; N, 5.71.

Synthesis of Partially Protected 2′-*O*-Methyluridylyl-(3′-5′)2′-*O*-methyldeoxyuridine Derivative 7a,b Having a Cyclic Structure with a Propylene Bridge. The fully protected dimer **6a,b** (721 mg, 0.748 mmol) was dissolved in pyridine (10.0 mL) and 25% aqueous ammonia (10.0 mL). After being stirred at room temperature for 3.5 h, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (70 g) with [$CHCl_3$−ether (4:1, v/v)]−MeOH (100:3, v/v) containing 1% pyridine to give DMTrUmpc3Um (fast isomer) **7a** (319 mg, 46%) and DMTrUmpc3Um (slow isomer) **7b** (298 mg, 43%) as foam. DMT-Umpc3Um (fast isomer) **7a**: TLC R_f 0.33 ($CHCl_3$:Et_2O:CH_3OH, 20:10:3, v/v/v, developed three times); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.70−1.95 (2H, m), 2.40−2.60 (2H, m), 3.45 (1H, m), 3.62−3.80 (15H, m), 3.88 (1H, d, $J_{2',3'}$ = 4.90 Hz), 4.15−4.27 (4H, m), 4.47 (1H, m), 4.68 (1H, m), 5.08 (1H, m), 5.23 (1H, d, $J_{5,6}$ = 7.90 Hz), 5.91 (1H, s), 6.10 (1H, d, $J_{1',2'}$ = 3.60 Hz), 6.84−6.87 (4H, m), 7.20−7.31 (9H, m), 7.80 (1H, s), 7.84 (1H, d, $J_{5,6}$ = 8.20 Hz), 9.70 (1H, br), 9.84 (1H, br); ^{13}C NMR (68 MHz, $CDCl_3$) δ 20.52, 26.81, 55.24, 58.60, 58.87, 61.33, 64.24, 65.68, 67.32, 73.77, 81.60, 82.10, 82.22, 82.41, 83.36, 86.45, 87.26, 87.48, 102.77, 110.24, 113.30, 127.44, 128.05, 128.25, 130.23, 134.45, 134.61, 137.34, 139.52, 143.63, 150.04, 150.60, 158.83, 163.14, 163.58; ^{31}P NMR (109 MHz, $CDCl_3$) δ −2.29. Anal. Calcd for $C_{44}H_{49}N_4O_{16}P\cdot H_2O$: C, 56.28; H, 5.48; N, 5.96. Found: C, 56.21; H, 5.45; N, 5.89. DMT-Umpc3Um (slow isomer) **7b**: TLC R_f 0.22 ($CHCl_3$:Et_2O:CH_3OH, 20:10:3, developed three times); ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.88−2.09 (2H, m), 2.35−2.57 (2H, m), 3.50−4.32 (23H, m), 5.09 (1H, m), 5.25 (1H, d, $J_{5,6}$ = 7.90 Hz), 5.93 (1H, s), 6.02 (1H, d, $J_{1',2'}$ = 2.30 Hz), 6.84−6.87 (4H, m), 7.24−7.34 (9H, m), 7.82 (1H, s), 7.94 (1H, d, $J_{5,6}$ = 8.30 Hz), 9.80 (2H, br); ^{13}C NMR (68 MHz, $CDCl_3$) δ 20.90, 27.04, 55.27, 58.65, 58.78, 60.72, 63.45, 66.05, 67.35, 73.71, 81.04, 81.18, 81.92, 82.03, 82.28, 83.50, 87.22, 87.37, 102.55, 110.37, 113.37, 127.42, 128.10, 128.30, 130.26, 130.33, 134.61, 134.68, 137.64, 139.62, 143.79, 150.13, 150.37, 158.79, 158.83, 163.43, 163.66; ^{31}P NMR (109 MHz, $CDCl_3$) δ −1.67. Anal. Calcd for $C_{44}H_{49}N_4O_{16}P\cdot H_2O$: C, 56.28; H, 5.48; N, 5.96. Found: C, 56.22; H, 5.48; N, 5.96.

Unprotected 2′-*O*-Methyluridylyl(3′-5′)2′-*O*-methyldeoxyuridine Derivative 8a,b Having a Cyclic Structure with a Propylene Bridge. Compound **7a** or **7b** (55 mg, 60 μmol) was dissolved in 80% acetic acid (2 mL). After being kept at room temperature for 30 min, the mixture was evaporated under reduced pressure. The residue was partitioned between ether (2.5 mL) and water (2.5 mL). The aqueous layer was collected, washed two times with ether, and lyophilized to give **8a** (30 mg, 82%) or **8b** (29 mg, 79%). Umpc3Um (fast isomer) **8a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.68−1.71 (2H, m), 2.32−2.35 (2H, m), 3.40 (3H, s), 3.46 (3H, s), 3.63 (2H, m), 3.86 (1H, dd, $J_{1',2'}$ = 1.7 Hz, $J_{2',3'}$ = 5.0 Hz), 3.88−3.94 (1H, m), 4.02−4.06 (2H, m), 4.12−4.17 (2H, m), 4.21−4.26 (2H, m), 4.45−4.49 (1H, m), 4.97 (1H, seven, $J_{2',3'}$ = 4.90 Hz, $J_{3',4'}$ = 2.80 Hz, $J_{3',P}$ = 7.50 Hz), 5.30−5.36 (2H, m), 5.72 (1H, dd, $J_{5,6}$ = 8.1 Hz, $J_{5,NH}$ = 2.2 Hz), 5.83 (1H, d, $J_{1',2'}$ = 1.70 Hz,), 5.90 (1H, d, $J_{1',2'}$ = 6.60 Hz), 7.55 (1H, s), 7.88 (1H, d, $J_{5,6}$ = 8.10 Hz), 11.36 (1H, br), 11.44 (1H, d, J_{NH-5H} = 2.2 Hz); ^{13}C NMR (68 MHz, DMSO-d_6) δ 20.27, 26.51, 26.65, 49.19, 57.86, 60.49, 64.85, 65.09, 67.59, 74.47, 74.54, 80.38, 81.33, 81.46, 82.86, 83.65, 85.50, 87.01, 102.46, 109.42, 137.41, 140.29, 150.19, 150.59, 162.89, 163.34; ^{31}P NMR (109 MHz, DMSO-d_6) δ −0.98. Anal. Calcd for $C_{23}H_{31}N_4O_{14}P\cdot H_2O$: C, 43.40; H, 5.23; N, 8.80. Found: C, 43.51; H, 5.23; N, 8.44. Umpc3Um (slow isomer) **8b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.62−1.80 (2H, m), 2.25−2.30 (1H, m), 2.36−2.41 (1H, m), 3.37 (3H, s), 3.47 (3H, s), 3.63 (2H, m), 3.85 (1H, d, $J_{2',3'}$ = 4.9 Hz), 3.93−3.97 (1H, m), 4.03−4.05 (2H, m), 4.10 (1H, t, $J_{1',2'}$ = $J_{2',3'}$ = 4.90 Hz), 4.18−4.25 (3H, m), 4.34−4.37 (1H, m, $J_{5',5''}$ = 11.80 Hz), 4.96 (1H, seven, $J_{2',3'}$ = 4.80 Hz, $J_{3',4'}$ = 2.50 Hz, $J_{3',P}$ = 7.10 Hz), 5.32−5.37 (2H, m), 5.72 (1H, d, $J_{5,6}$ = 8.10 Hz), 5.83 (1H, s), 5.90 (1H, d, $J_{1',2'}$ = 6.60 Hz), 7.65 (1H, s), 7.89 (1H, d, $J_{5,6}$ = 8.10 Hz), 11.33 (1H, br), 11.44 (1H, br); ^{13}C NMR (68 MHz, DMSO-d_6) δ 20.53, 26.60, 26.71, 57.92, 60.51, 63.54, 65.34, 67.46, 75.19, 75.28,

80.54, 81.24, 81.37, 83.02, 83.79, 83.87, 85.20, 87.30, 102.48, 109.44, 137.18, 140.13, 150.15, 150.59, 162.89, 163.40; ^{31}P NMR (109 MHz, DMSO-d_6) δ −1.61. Anal. Calcd for $C_{23}H_{31}N_4O_{14}P\cdot1.5H_2O$: C, 42.79; H, 5.31; N, 8.68. Found: C, 42.89; H, 5.10; N, 8.63.

Synthesis of Fully Protected 2′-*O*-Methyluridylyl(3′-5′)2′-*O*-methyldeoxyuridine Phosphorothioate Derivative 9a,b Having a Cyclic Structure with a Propylene Bridge. A mixture of **5** (179 mg, 0.5 mmol) and 1*H*-tetrazole (210 mg, 3.0 mmol) was rendered anhydrous by coevaporation with dry toluene, and the residue was dissolved in dry acetonitrile (25 mL) and dioxane (25 mL). A solution of **4** (0.5 mmol) in acetonitrile (2.0 mL) was added dropwise to the mixture over a period of 5 min. The resulting mixture was stirred at room temperature for 4 h and a solution of **4** (0.25 mmol) in CS_2 (1.0 mL) was added. After stirring was continued for an additional 5 h, the mixture was evaporated under reduced pressure. The residue was dissolved in acetonitrile (5 mL) and a solution of elemental sulfur (641 mg, 2.5 mmol) in acetonitrile (5.0 mL) was added to the mixture. The solution was stirred at room temperature for 3 h, filtered, and evaporated under reduced pressure. The residue was extracted with $CHCl_3$ (50 mL) and 5% $NaHCO_3$ (50 mL). The organic layer was collected, washed two times with 5% $NaHCO_3$ (25 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in pyridine (5 mL) and 25% aqueous ammonia (5 mL) was added. The mixture was stirred at room temperature for 3 h and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (25 g) with $CHCl_3$−MeOH (100:2, v/v) containing 1% pyridine to give DMT-Umspc3Um **9a,b** as foam (200 mg, 43%, the ratio of the diasteromers 1.1:1): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.74−2.00 (4H, m), 2.34−2.86 (4H, m), 3.43−4.35 (45H, m), 4.66−4.69 (1H, m), 5.20−5.32 (2H, m), 5.88 (1H, s, 1′−H of pU), 6.00 (1H, s), 6.04 (1H, d, $J_{1',2'}$ = 2.64 Hz), 6.12 (1H, d, $J_{1',2'}$ = 4.62 Hz), 5.99−6.04 (4H, m), 6.84−6.87 (8H, m), 7.25−7.41 (18H, m), 7.71 (1H, s), 7.82 (1H, d, $J_{5,6}$ = 8.25 Hz), 7.93 (1H, s), 8.01 (1H, d, $J_{5,6}$ = 7.91 Hz), 10.02−10.13 (4H, m); ^{13}C NMR (68 MHz, $CDCl_3$) δ 20.58, 20.88, 21.31, 26.11, 26.70, 54.65, 55.13, 58.51, 58.64, 58.80, 60.40, 61.69, 62.12, 63.00, 64.91, 65.84, 67.10, 67.78, 73.71, 74.68, 80.94, 81.74, 81.89, 82.19, 83.18, 83.49, 86.27, 87.21, 87.40, 102.50, 102.86, 109.90, 110.60, 113.23, 125.12, 127.24, 127.94, 128.10, 128.19, 128.86, 129.00, 130.06, 130.21, 134.43, 134.47, 134.56, 134.65, 137.47, 137.68, 139.46, 143.65, 143.68, 150.15, 150.39, 150.71, 158.63, 158.69, 163.38, 163.61, 163.76, 163.85; ^{31}P NMR (109 MHz, $CDCl_3$) δ 67.17, 68.80. Anal. Calcd for $C_{44}H_{49}N_4O_{15}PS\cdot2H_2O$: C, 54.31; H, 5.49; N, 5.76. Found: C, 54.00; H, 5.38; N, 5.82.

Unprotected 2′-*O*-Methyluridylyl(3′-5′)2′-*O*-methyldeoxyuridine Phosphorothioate Derivative 10a,b Having a Cyclic Structure with a Propylene Bridge. Compound **9a**,**b** (83.7 mg, 89.3 μmol) was dissolved in 80% acetic acid (2 mL). After being kept at room temperature for 30 min, the mixture was evaporated under reduced pressure. The residue was partitioned between ether (10 mL) and water (10 mL). The aqueous layer was collected, washed two times with ether, and lyophilized to give **10a,b** (52 mg, 91%): Anal. Calcd for $C_{23}H_{31}N_4O_{13}PS\cdot2H_2O$: C, 41.19; H, 5.26; N, 8.36. Found: C, 41.32; H, 5.02; N, 8.25. Reversed-phase HPLC of this mixture gave **10a** (365 A_{260}, 21%) and **10b** (396 A_{260}, 22%). Umspc3Um (fast isomer) **10a**: reversed-phase HPLC 17.4 min (system 1b); UV (H_2O−CH_3CN, 100:1, v/v) λ_{max} 263 nm, λ_{min} 232 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.58−1.88 (2H, m), 2.19−2.45 (2H, m), 3.44 (3H, s), 3.47 (3H, s), 3.57−3.65 (2H, m), 3.81 (1H, d, $J_{2',3'}$ = 4.50 Hz), 3.98−4.04 (2H, m), 4.05−4.15 (4H, m), 4.24−4.36 (2H, m), 5.06 (1H, ddd, $J_{2',3'}$ = 4.85 Hz, $J_{3',4'}$ = 2.25 Hz, $J_{3',P}$ = 10.86 Hz), 5.21−5.39 (2H, m), 5.72 (1H, d, $J_{5,6}$ = 8.09 Hz), 5.76 (1H, s), 5.89 (1H, d, $J_{1',2'}$ = 6.85 Hz), 7.70 (1H, s), 7.88 (1H, d, $J_{5,6}$ = 8.14 Hz), 11.32 (1H, br), 11.44 (1H, br); ^{13}C NMR (68 MHz, DMSO-d_6) δ 20.74, 26.51, 26.63, 57.99, 60.61, 63.81, 65.71, 67.32, 76.25, 79.21, 80.52, 81.15, 81.30, 83.08, 83.97, 85.34, 87.59, 102.63, 109.17, 137.02, 140.20, 150.14, 150.68, 162.97, 163.56; ^{31}P NMR (109 MHz, DMSO-d_6) δ 67.04. Umspc3Um (slow isomer) **10b**: Reversed-phase HPLC: 19.4 min (system 1b); UV (H_2O−CH_3CN, 100:1, v/v) λ_{max} 261 nm, λ_{min} 231 nm; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.60−1.82 (2H, m), 2.28−2.43 (2H, m), 3.38 (3H, s), 3.41 (3H, s), 3.58−3.65 (2H, m), 3.82 (1H, m, $J_{1',2'}$ = 1.22 Hz, $J_{2',3'}$ = 5.03 Hz), 4.02−4.08 (2H, m), 4.12−4.17 (3H, m), 4.29 (1H, m, $J_{2',3'}$ = 5.18 Hz, $J_{3',P}$ = 7.41 Hz), 4.53 (1H, ddd, $J_{4',5'}$ = 2.29 Hz, $J_{5',5''}$ = 9.51 Hz, $J_{5',P}$ = 6.71 Hz, 5.34−5.39 (2H, m), 5.72 (1H, d, $J_{5,6}$ = 8.09 Hz), 5.89 (1H, d, $J_{1',2'}$ = 1.56 Hz), 5.90 (1H, d, $J_{1',2'}$ = 6.87 Hz), 7.44 (1H, s), 7.87 (1H, d, $J_{5,6}$ = 8.14 Hz), 11.35 (1H, br), 11.43 (1H, br); ^{13}C NMR (68 MHz, DMSO-d_6) δ 20.54, 26.35, 26.47, 57.97, 60.70, 65.88, 68.22, 75.64, 79.18, 80.27, 81.03, 81.17, 83.06, 83.74, 85.48, 86.92, 102.68, 109.74, 137.74, 140.33, 150.26, 150.71, 162.98, 163.33; ^{31}P NMR (109 MHz, DMSO-d_6) δ 67.90.

Synthesis of 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-methyluridylyl(3′-5′)2′-*O*-methyluridine Phosphoramidite Derivative (11a,b). DMT-Umpc3Um (fast isomer) (**7a**) (230 mg, 0.25 mmol) was rendered anhydrous by repeated coevaporations with dry toluene and finally dissolved in CH_2Cl_2 (2.5 mL). To the mixture were added triethylamine (105 μL, 0.75 mmol) and 2-cyanoethoxydiisopropylaminochlorophosphine (89 mg, 0.375 mmol). The mixture was stirred at room temperature for 2 h and then quenched by addition of ethanol (0.5 mL). The mixture was diluted with $CHCl_3$ (20 mL). The $CHCl_3$ solution was washed three times with 5% $NaHCO_3$ (20 mL), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (2 mL) and this solution was added portionwise to hexane (100 mL)−ether (30 mL)−pyridine (1.5 mL) with vigorous stirring. The resulting white precipitate was collected by removal of the solvent by a pipet and dried in vacuo to give DMTrUmpc3Ump(a,ce) (fast isomer) (**11a**) (241 mg, 86%). In a similar manner, from **7b** (230 mg, 0.25 mmol) DMTrUmpc3Ump(a,ce) (slow isomer) (**11b**) (271 mg, 97%) was obtained. DMTrUmpc3Ump(a,ce) (fast isomer) (**11a**): ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.14−1.28 (24H, m), 1.60−1.81 (4H, m), 2.36−2.64 (8H, m), 3.40−4.40 (48H, m), 4.69−4.75 (2H, m), 4.96−5.07 (2H, m), 5.17−5.24 (1H, m), 5.88−5.95 (2H, 2s), 6.02−6.06 (2H, m), 6.81−6.88 (8H, m), 7.17−7.37 (18H, m), 7.70−7.73 (2H, 2s), 7.86−7.94 (2H, 2d, $J_{5,6}$ = 8.40 Hz), 8.19 (4H, br); ^{13}C NMR (68 MHz, $CDCl_3$) δ 20.25, 24.35, 24.46, 27.05, 42.98, 43.16, 55.08, 57.58, 57.94, 58.22, 58.42, 58.64, 60.47, 61.13, 63.70, 65.48, 68.29, 68.50, 68.81, 72.11, 80.52, 82.30, 82.55, 83.04, 86.85, 87.01, 87.17, 87.39, 88.23, 102.28, 110.08, 110.24, 113.10, 117.52, 117.77, 127.22, 127.87, 128.03, 129.99, 134.43, 134.54, 139.26, 143.65, 150.28, 158.65, 163.49, 163.90; ^{31}P NMR (109 MHz, $CDCl_3$) δ −2.00, −1.09, 149.89, 151.11. MS (FAB^+) calcd for $C_{53}H_{67}N_6O_{17}P$ (M + H) 1121.4038, found 1121.4049. DMTrUmpc3Ump(a,ce) (slow isomer) (**11b**): ^{1}H NMR (270 MHz, $CDCl_3$, TMS) δ 1.17−1.26 (24H, m), 1.70−2.01 (4H, m), 2.38−2.83 (8H, m), 3.44−4.33 (54H, m), 5.09−5.19 (2H, m), 5.22−5.26 (2H, m), 5.95−6.02 (4H, m), 6.84−6.88 (8H, m), 7.21−7.32 (18H, m), 7.68−7.74 (2H, 2s), 7.90−7.96 (2H, m), 8.53 (4H, br); ^{13}C NMR (68 MHz, $CDCl_3$) δ 20.02, 20.11, 20.38, 24.35, 24.46, 26.83, 42.97, 43.15, 54.93, 57.81, 58.11, 58.29, 58.44, 60.27, 62.63, 65.66, 68.47, 68.64, 73.26, 80.54, 80.68, 82.10, 83.15, 86.97, 88.05, 102.28, 110.37, 110.53, 113.03, 117.59, 118.04, 127.08, 127.78, 128.03, 130.03, 134.29, 134.45, 136.96, 143.55, 150.31, 158.54, 163.49, 163.81; ^{31}P NMR (109 MHz, $CDCl_3$) δ −3.27, −3.06, 150.45, 151.26. MS (FAB^+) calcd for $C_{53}H_{67}N_6O_{17}P$ (M + H) 1121.4038, found 1121.4010.

Typical Procedure for the Synthesis of Oligonucleotides Incorporating Umc3Um in the Solid-Phase Approach. The standard protocol described in the ABI manual for ABI 381 synthesizer was used. For the incorporation of Umpc3Um into the middle position of dodecaoligonucleotides, the Umpc3Um amidite was used in a glass vessel with a filter. A T-loaded CPG resin (1 μmol, 44 mmol/g, Glen Research, 500 Å) or U-loaded CPG resin (1 μmol, 19.7 μmol/g, Glen Research, 500 Å) was used. When the modified dimer is inserted, the following manual manipulation procedure was used: (1) detritylation with 3% TCA/CH_2Cl_2, (2) washing with pyridine, (3) drying, CH_3CN for 10 min, (4) condensation with the amidite unit (0.1 M, 20 equiv) in the presence of 1*H*-tetrazole (0.5 M,100 equiv)/CH_3CN for 10 min, (5) washing with CH_3-

CN, (6) capping with Ac_2O-Py (1:9, v/v) in the presence of 0.1 M DMAP for 1 min, (7) washing with pyridine, (8) oxidation with 0.05 M I_2/THF−Py−H_2O for 1 min (7:2:1, v/v/v), (9) washing with pyridine, (10) washing with CH_2Cl_2. After the final condensation was finished, the CPG gel was removed from the synthesizer and treated with 25% NH_3 aq-Py (9:1, v/v, 2 mL) for 20 h. The excess ammonia was removed under reduced pressure, and the resin was suspended in water (1 mL). The mixture was evaporated under reduced pressure and lyophilized. (When the synthesis of RNA oligomers was carried out, the resin was treated with 1 M TBAF·H_2O in THF (1 mL) for 16 h and thereafter desalting was performed by gel filtration using Sephadex G-15. The eluate was lyophilized.) The lyophilized material was separated by anion-exchange HPLC using a FAX column followed by reversed-phase HPLC: U_4[Umpc3Um(fast)]U_4 (**13a**) (average yield 98%), 19.9 A_{260} (22%) (ϵ 9.27 × 10^3); U_4[Umpc3Um(slow)]U_4 (**13b**) (average yield 98%), 16.5 A_{260} (18%) (ϵ 9.27 × 10^3); T_4[Umpc3Um-(fast)]T_4 (**15a**) (average yield 99%), 44.2 A_{260} (58%) (ϵ 7.65 × 10^3); T_4[Umpc3Um(slow)]T_4 (**15b**) (average yield 99%), 18.9 A_{260} (25%) (ϵ 7.65 × 10^3).

Enzymatic Assay of Oligonucleotides Incorporating Umpc3Um. A modified oligonucleotide (1.0 A_{260}) was dissolved in Tris−HCl buffer (80 μL, pH 8.0) and snake venom phosphodiesterase (1 unit/μL, 8 μL). The mixture was incubated at 37 °C for 12 h and then calf intestinal alkaline phosphatase (4 unit/mL, 5 μL) was added. The mixture was incubated at 37 °C for 6 h, and an aliquot was analyzed by reversed-phase HPLC.

Structural Analysis of *R*p-Umpc3Um and *S*p-Umpc3Um by Molecular Mechanics. MacroModel ver 6.0 was used in a Silicon Graphics O2 workstation by using the AMBER* force field. The PRCG program and GB/SA model were used for energy minimization. Many conformers were generated using 5000 steps by Monte Carlo method and energy-minimized.

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