JOC
The Journal of Organic Chemistry

VOLUME 65, NUMBER 12 JUNE 16, 2000

Articles

Synthesis and Properties of Oligodeoxynucleotides Incorporating a Conformationally Rigid Uridine Unit Having a Cyclic Structure at the 5′-Terminal Site

Mitsuo Sekine,* Osamu Kurasawa, Koh-ichiroh Shohda, Kohji Seio, and Takeshi Wada

Department of Life Science, Tokyo Institute of Technology, Nagatsuta, Midoriku, Yokohama 226-8501, Japan

Received August 30, 1999

A 2′-*O*-methyluridylic acid derivative **3** having a cyclic structure linked between the 5-position of the uracil residue and the 5′-phosphate group was synthesized. The NMR analysis suggests that this cyclouridylic acid derivative has exclusively the C3′-endo conformation that is in favor of duplex formation with RNA. Two oligonucleotides [pc3Um(pT)$_9$ and pc3Um(pU)$_9$] incorporating this cyclouridylic acid unit at the 5′-terminal site were synthesized by using the fully protected cyclouridylic acid 3′-phosphoramidite derivative **11** in the solid-phase synthesis. To examine the actual effect of this cyclic structure on the thermal stability of duplexes between the modified oligonucleotides and their complementary oligonucleotides, two oligonucleotides [pUm(pT)$_9$ and pUm(pU)$_9$] having an acyclic structure were also synthesized. As the complementary oligonucleotides, dA(pdA)$_9$ and A(pA)$_9$ were used for T_m experiments with these 5′-terminal modified oligonucleotides. The T_m values of all the possible duplexes were measured. These results clearly show that the duplex of pc3Um(pT)$_9$−A(pA)$_9$ has a higher T_m value by 5.5 °C than that of A(pA)$_9$−T(pT)$_9$. This is rather significant compared with all other cases. Moreover, the T_m value of pc3Um-(pT)$_9$−A(pA)$_9$ is 4.5 °C higher than that of pUm(pT)$_9$−A(pA)$_9$. This result suggests that the cyclic structure can considerably contribute to stabilization of the duplex only in the case of the modified oligomer (*DNA) and decaadenylate (RNA).

Introduction

Introduction of cyclic structures into nucleosides has proved to be a powerful tool for conformational studies of the sugar puckering and the glycoside torsion angle of nucleosides and nucleotides.[1] Recently, much attention has been paid to the synthesis of oligonucleotides incorporating a cyclic structure in connection with recent developments of the antisense/antigene strategy.[2] Particularly, the chemical fixation of the ribose moiety with three- to five-membered rings has been applied to the synthesis of conformationally rigid oligonucleotides.[3−12]

(1) (a) Maruyama, T.; Adachi, Y.; Honjo, M. *J. Org. Chem.* **1988**, *53*, 4552−4555. (b) Yoshimura, Y.; Otter, B. A.; Ueda, T.; Matsuda, A. *Chem. Pharm. Bull.* **1992**, *40*, 1761−1769.

(2) For comprehensive reviews, see: Sanghvi, Y. S.; Cook, P. D., Eds. In *Carbohydrate Modifications In Antisense Research*; American Chemical Society: Washington, DC, 1994. Knorre, D. G.; Vlassov, V. V.; Zarytova, V. F.; Lebedev, A. V.; Federova, O. S. In *Design and Targeted Reactions of Oligonucleotide Derivatives*; CRC Press: Boca Raton, 1994. Agrawal, A., Ed. In *Antisense Therapeutics*; Humana Press: Totowa, 1996. Chadwick, D. J.; Cardew, G., Eds. In *Oligonucleotides as Therapeutic Agents*; John Wiley & Sons: New York, 1997. Stein, C. A.; Krieg, A. M., Eds. In *Applied Antisense Oligonucleotides Technology*; Wiley-Liss: New York, 1998.

10.1021/jo991374h CCC: $19.00
Published on Web 05/18/2000

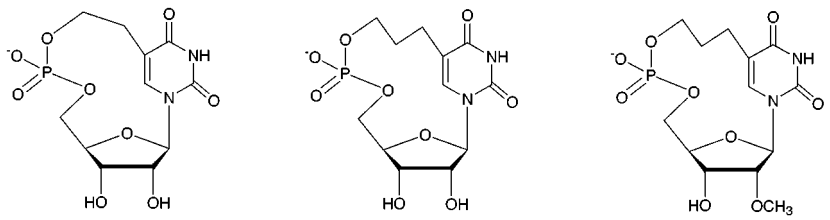


Figure 1. Intramolecularly cyclized 5′-uridylic acid derivatives.

More recently, we have reported the fixation of 5′-uridylic acid in a C3′-endo conformation by introduction of covalent bond linkers between the 5-position of the uracil ring and the 5′-phosphate group.[13] We came up with this idea originally from the unique structure of an intramolecular hydrogen bond of 5-[(methylamino)methyl]-2-thiouridine 5′-phosphate which was discovered as the first letter of the anticodon in *Escherichia coli* $tRNA^{Arg}$ and suggested to exist in a C3′-endo conformation.[14] Therefore, we have synthesized 5′-cyclouridylic acid derivatives such as compounds **1** (pc2U) and **2** (pc3U) as shown in Figure 1 (where c2 and c3 refer to the ethylene and propylene bridge between the 5′-phosphate and the 5-position of the uracil residue).

Actually, the intramolecular cyclic structure of **2** having a propylene linker was found to be an essential factor for stabilization of the C3′-endo conformation.[13b,c] This covalent-bonding linker also resulted in the simultaneous fixation of the anti-oriented base and the g^+ torsion angle around the C4′−C5′ bond, which are seen in the typical A-type RNA helix.[13] The ^{1}H NMR analysis of **2** suggested that the C3′-endo conformation exists in more than 88%.[13b,c]

In this paper, we report the chemical synthesis of oligonucleotides incorporating a cyclouridylic acid derivative **3** (pc3Um, where Um refers to 2′-*O*-methyluridine) masked with a methyl group at the 2′-position into the 5′-terminal site and marked effects of their duplexes with complementary oligonucleotides on the thermal stability.

Results and Discussion

A 2′-*O*-methylated cyclic uridylic acid derivative **10** was chosen as the starting material to introduce a cyclic structure into the 5′-terminal site of decathymidylate $T(pT)_9$ or decauridylate $U(pU)_9$. This 2′-*O*-methylated species enables us to circumvent the tedious technical problem arising from the necessity of the selective protection of the 2′-hydroxyl group of **2** for chain elongation. It is also known that the 2′-*O*-methyl group in ribonucleoside derivatives affects the sugar puckering in favor of a C3′-endo conformation because of the steric repulsion between the 2-keto group and the methyl group.[15] Compound **10** was synthesized via several steps from 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-methyluridine (**4**).[16] The 3′-*O*-acetylation of **4** with acetic anhydride followed by treatment with 80% acetic acid gave the 3′-*O*-acetyluridine derivative **5** in 86% yield. Iodination of **5** with ICl[17] in dioxane gave the 5-iodouridine derivative **6** in 81% yield. The reaction of **6** with propargyl alcohol in the presence of palladium tetrakis(triphenylphosphine)/CuI/Et_3N[17] resulted in the 5-substituted product **7** in 57% yield. The Pd-catalyzed hydrogenation of **7** gave the diol derivative **8** in 91% yield.

(3) (a) Rodriguez, J. B.; Marquez, V. E.; Micklaus, M. C.; Barchi, J. J., Jr. *Tetrahedron Lett.* **1993**, *34*, 6233−6236. (b) Rodriguez, J. B.; Marquez, V. E.; Nicklaus, M. C.; Mitsuya, H.; Barchi, J. J., Jr. *J. Med. Chem.* **1994**, *37*, 3389−3399. (c) Ezzitouni, A.; Barchi, J. J., Jr.; Marquez, V. E.; *J. Chem. Soc., Chem. Commun.* **1995**, 1345−1346. (d) Jeong, L. S.; Marquez, V. E.; Yuan, C.-S.; Borchardt, R. T. *Heterocycles* **1995**, *41*, 2651−2656. (e) Siddiqui, M. A.; Ford, H., Jr.; George, C.; Marquez, V. E. *Nucleosides Nucleotides* **1996**, *15*, 235−250. (f) Jeong, L. S.; Marquez, V. E.; *Tetrahedron Lett.* **1996**, *37*, 2353−2356. (g) Marquez, V. E.; Siddiqui, M. A.; Ezzitouni, A.; Russ, P.; Wang, J. Wagner, R. W.; Matteucci, M. D. *J. Med. Chem.* **1996**, *39*, 3739−3747. (h) Ezzitouni, A.; Marquez, V. E. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1073−1078. (i) Ezzitouni, A.; Russ, P.; Marquez, V. E. *J. Org. Chem.* **1997**, *62*, 4870−4873.

(4) (a) Tarköy, M.; Bolli, M.; Schweizer, B.: Leumann, C. *Helv. Chim. Acta* **1993**, *76*, 481−510.(b) Tarköy, M.; Leumann, C. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1432−1434. (c) Tarköy, M.; Bolli, M.; Leumann, C. *Helv. Chim. Acta* **1994**, *77*, 716−744. (d) Litten, C. J.; Epple, C.; Leumann, C. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1231−1234. (e) Bolli, M.; Lubini, P.; Leumann, C. *Helv. Chim. Acta* **1995**, *78*, 2077−2096. (d) Boll, M.; Litten, C.; Schütz, R.; Leumann, C. *Chem. Biol.* **1996**, *3*, 197−206. (f) Litten, J. C.; Leumann, C. *Helv. Chim. Acta* **1996**, *79*, 1129−1146. (g) Bolli, M.; Trafelet, H. U.; Leumann, C. *Nucleic Acids Res.* **1996**, *24*, 4660−4667. (h) Steffens, R.; Leumann, C. J. *Helv. Chim. Acta* **1997**, *80*, 2426−2439. (i) Steffens, R.; Leumann, C. J. *J. Am. Chem. Soc.* **1997**, *119*, 11548−11549. (j) Steffens, R.; Leumann, C. J. *J. Am. Chem. Soc.* **1999**, *121*, 3251−3255.

(5) Jones, R. J.; Swaminathan, S.; Milligan, J. F.; Wadwani, S.; Froehler, B. C.; Matteucci, M. D. *J. Am. Chem. Soc.* **1993**, *115*, 9816−9817. (c) Zou, R.; Matteucci, M. D. *Tetrahedron Lett.* **1996**, *37*, 941−944.

(6) Bevierre, M.-O.; De Mesmaeker, A.; Wolf, R. M.; Freier, S. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 237−240.

(7) 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.

(8) Herdewijn, P. *Liebigs Ann.* **1996**, 1337−1348.

(9) Egli, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1894−1909.

(10) (a) Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735−8738. (b) Obika, S.; Morio, K.; Nanbu, D.; Imanishi, T. *Chem. Commun.* **1997**, 1643−1644. (c) Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401−5404.

(11) (a) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *J. Chem. Soc., Chem. Commun.* **1998**, 455−456. (b) 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. (c) Christensen, N. K.; Petersen, M.; Nielsen, P.; Jacobsen, J. P.; Olsen, C. E.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 5458−5463.

(12) Wang, G.; Gunic, E.; Girardet, J.-L.; Stoisavljevic, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1147−1150.

(13) (a) Seio, K.; Wada, T.; Sakamoto, K.; Yokoyama, S.; Sekine M. *J. Org. Chem.* **1996**, *61*, 1500−1504. (b) Seio, K.; Wada, T.; Sakamoto, K.; Yokoyama, S.; Sekine M. *Tetrahedron Lett.* **1995**, *36*, 9515−9518. (c) Seio, K.; Wada, T.; Sakamoto, K.; Yokoyama, S.; Sekine M. *Nucleosides Nucleotides* **1997**, *16*, 1023−1032.

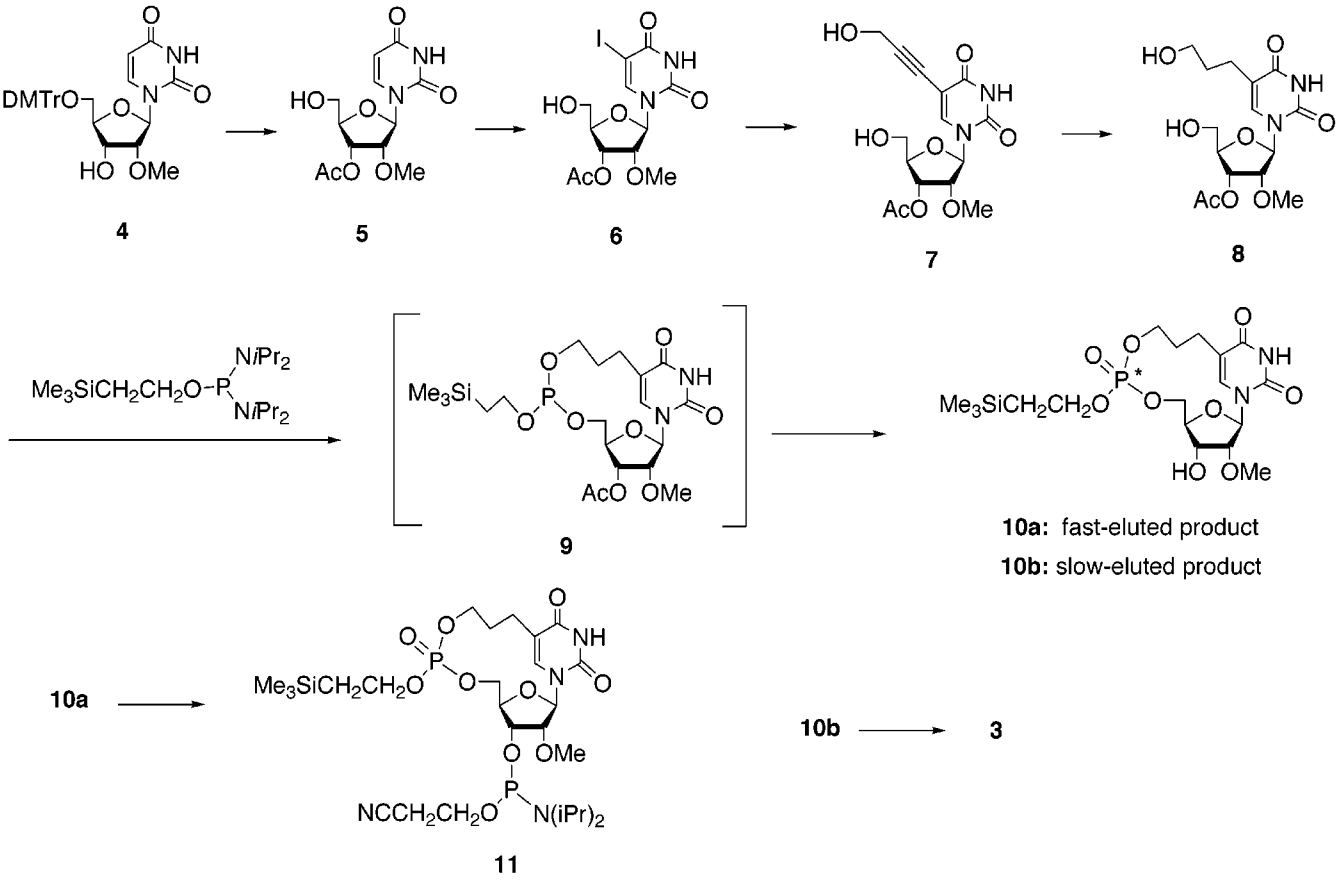
(14) (a) Sakamoto, K.; Kawai, G.; Niimi, T.; Watanabe, S.; Niimi, T.; Satoh, T.; Sekine, M.; Yamauti, Z.; Nisimura, S.; Miyazawa, T.; Yokoyama, S. *Eur. J. Biochem.* **1993**, *216*, 369−375. (b) Sakamoto, K.; Kawai, G.; Watanabe, S.; Niimi, T.; Hayasi, N.; Muto, Y.; Watanabe, K.; Satoh, T.; Sekine,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

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

(16) Inoue, H.; Hayase, Y.; Imura, A.; Iwai, S.; Miura, K.; Ohtsuka E. *Nucleic Acids Res.* **1987**, *15*, 6131−6148.

(17) (a) Robins M. J.; Barr, P. J. *J. Org. Chem.* **1983**, *48*, 1854−1862. (b) Hobbs, F. W., Jr. *J. Org. Chem.* **1989**, *54*, 3420−3422.

Scheme 1



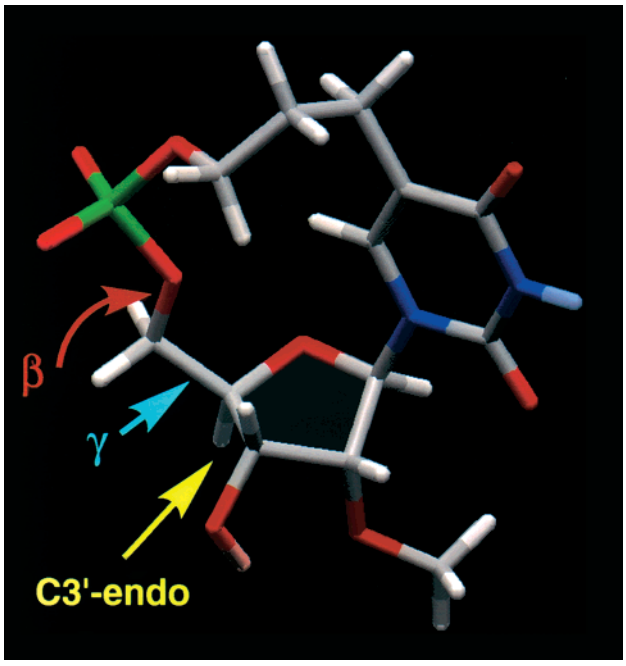


Figure 2. The most stable conformer energy-minimized by molecular mechanics calculation.

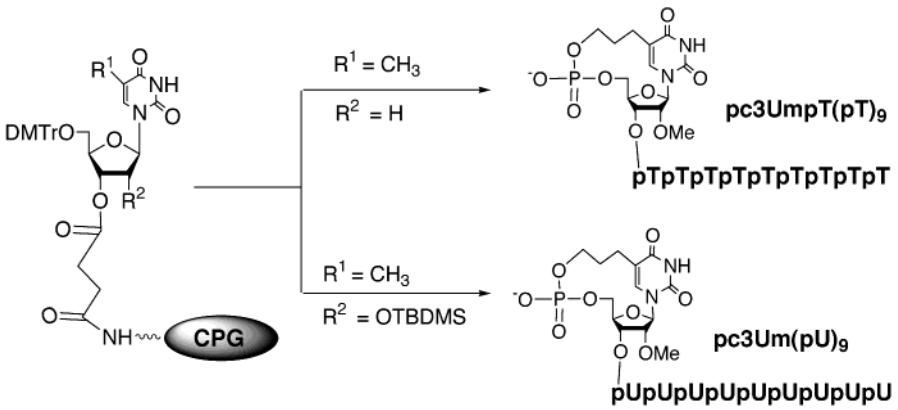
Reaction of **8** with bis(diisopropylamino)[2-(trimethylsilyl)ethoxy]phosphine[18] in the presence of 1*H*-tetrazole[19] gave the cyclic phosphite intermediate **9**, which in situ was converted by the successive treatments with *t*-BuOOH[20] and ammonia to the desired cyclic uridylic acid derivative **10** in an overall yield of 69%. The trimethylsilylethyl group was found to be sufficiently stable during the treatment with ammonia so that the selective deacetylation was achieved from **7**. The product **10** was formed as a set of diasteroisomers in ca. 1:1 ratio due to the chirality of the phosphorus center. The fast-eluted product **10a** and the slow-eluted product **10b** were successfully separated by silica gel column chromatography and isolated in 34 and 35% yields, respectively.

The stereoisomer **10a** was used to synthesize the 3′-terminal phosphoramidite unit **11**, while **10b** was deprotected by treatment with TBAF·H_2O to give the unprotected cyclic uridylic acid **3**. This trimethylsilylethyl group could be easily removed under the conditions prescribed for removal of the 2′-*O*-TBDMS group in the standard RNA synthesis.[21] Actually, conversion of **10b** to **3** required 2 h, which is shorter than that for the 2′-*O*-TBDMS group.

Conformational Analysis of Cyclouridylic Acid 3. The ^{1}H NMR spectrum of **3** suggests that this compound exists exclusively in a C3′-endo conformation since the 1′-proton was observed as a doublet near a singlet in 400 MHz NMR. The computer simulation of this peak give 1.34 Hz as the $J_{1',2'}$ coupling constant. The $J_{3',4'}$ value was 8.1 Hz. It is also known that the average value of the sum of $J_{1',2'} + J_{3',4'}$ in uridine derivatives is 9.4 Hz ± 0.2

(18) Wada, T.; Sekine, M. *Tetrahedron Lett.* **1994**, *35*, 757−760.
(19) Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185−3191.
(20) Engels, J.; Jäger, A. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 2−913.
(21) Beaucage, S. L. In *Protocols for Oligonucleotides and Analogs, Synthesis and Properties*; Agrawal, S., Ed.; Humana Press: Totowa, NJ, 1993; pp 33−61.

Scheme 2



Hz.[22] Actually, the sum of $J_{1',2'} + J_{3',4'}$ in **2** is 9.4 Hz.[13b] From the above J coupling constants observed, the sum of $J_{1',2'} + J_{3',4'}$ in **3** is calculated to be ≤9.4 Hz. The fractional population of the 3′-endo conformer of **3** was calculated to be 86% according to the well-known equation [P(C3′-endo) = $J_{3',4'}/(J_{1',2'} + J_{3',4'})$] reported by Altona.[23] The $J_{p,5'}$ and $J_{p,5''}$ values observed were 2.2 and 4.4 Hz, respectively. From these values, the percentage of the β^t confomer around the C5′−O bond was 92%, which was calculated by the equation of $P(\beta^t) = 100 \times [25.5 - (J_{p,5'} + J_{p,5''})]/20.5$.[24] This β^t conformer population is rather high apparently due to the cyclic structure. The fractional polulation of the g^+ conformer around the $C_{4'-5'}$ bond was calculated to be 94% by the equation of $P(g+) = [13.3 - (J_{4',5'} + J_{4',5''}]/9.7$.[25] In addition, we observed the J coupling between the phosphorus and the β-carbon of the propylene bridge, which was measured to be 9.2 Hz. According to the equation of $J = 8.0 \cos\theta^2 - 3.4 \cos\theta + 0.5$,[26] the torsion angle around the PO−CC bond was estimated to be 148°. Since the J coupling between the 4′C and the phosphorus was also 9.2 Hz, a similar dihedral angle can be estimated for PO−CC(4′). These values are quite reasonable judging from the 3D-model, which was obtained by molecular mechanics calculation.

More interestingly, it turned out that the three methylene groups of the linker have clear geminal couplings with 10.1, 14.5, and 14.7 Hz. In addition, the W-shape type long-range J cupling (^4J = 3.1 Hz) between the phosphorus and the proton on the β-carbon was observed. Furthermore, the NOE experiments disclosed that the 6-H proton interacts with one of the protons on the α-carbon of the propylene bridge and more strongly correlated with the 3′-proton than the 2′-proton. These facts strongly indicate that the propylene bridge is almost completely fixed in a rigid conformation. The molecular mechanics calculation also supported this observation as shown in Figure 2. The CD spectra of **3** in phosphate buffer (pH 7.0) at 25, 50, 80 °C were measured with those of pU as shown in Supporting Information. The Cotton effect of **3** around 270 nm was affected somewhat more significantly than that of pU with increasing temperature. The θ value at the positive peak in **3** at 25 °C is 15% greater than that of pU. At 80 °C, the θ value of **3** reaches the same level as that of pU.

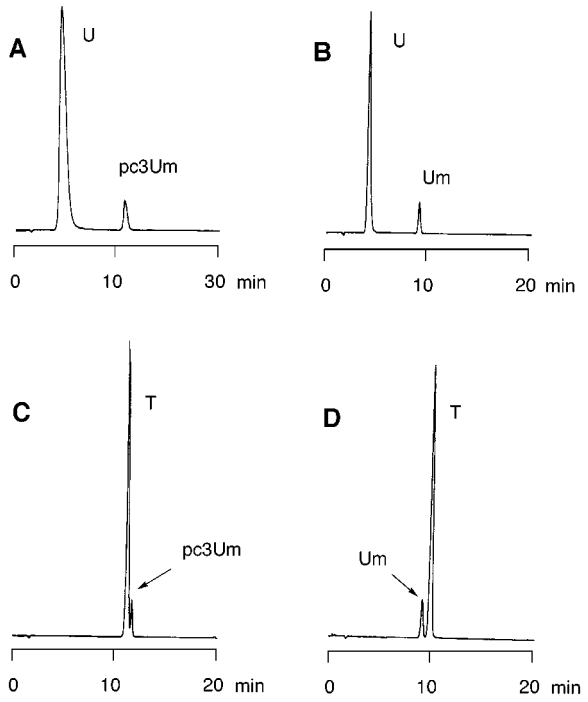


Figure 3. Enzymatic analysis of $pc3UmU_9$ (A), $pUmU_9$ (B), $pc3UmT_9$ (C), $pUmT_9$ (D) by digestion with snake venom phosphodiesterase followed by alkaline phosphatase.

Compound **3** was stable under basic conditions such as concd ammonia for 1 day, which are compatible with the standard conditions required for the current DNA synthesis. It was also confirmed that the cyclouridylic acid **3** was resistant to snake venom phosphodiesterase, calf spleen phosphodiesterase, and nuclease P1 for 24 h. Since the predominance of the C3′-endo conformation and the chemical stability in **3** were ascertained, oligonucleotides incorporating the structure unit of **3** were synthesized by an automated synthesizer using the phosphoramidite block **11**.

Two oligonucleotides having the unit **3** were synthesized on CPG gel as shown in Scheme 2. One is $pc3Um(pT)_9$ and the other is $pc3Um(pU)_9$, where pc3Um refers to the structure of **3**. The standard protocol was used for the chain elongation cycle.[27] Under the condi-

(22) Davis, D. B. *Prog. Nucl. Magn. Reson. Spectrosc.* **1978**, *12*, 135−225.
(23) Altona, C.; Sundaralingham, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333−2344.
(24) Lankhorst, P. P.; Hassnoot, C. A. G.; Erkelens, C.; Altona, C. *J. Biomol. Struct. Dyn.* **1984**, *1*, 1387−1405.
(25) Altona, C. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 413−433.
(26) Wijmenga, S. S.; Mooren, M. M. W.; Hilbers, C. W. In *NMR of Macromolecules*; Roberts, G. C. K., Ed.; IRL Press: Oxford, 1993; pp 217−288.
(27) Horn, T.; Urdea, M. S. *Tetrahedron Lett.* **1986**, *27*, 4705−4708.

Table 1. Summary of Thermal Stability of Duplixes between Modified or Natural Oligouridylate and DNA or RNA Oligoadenylate

Modified Oligomer \ Target Oligomer	d(ApApApApApApApApA) DNA (target)			ApApApApAApApApA RNA (target)			ApApApApAApApApG RNA (mismatched)	
		Tm	⊿Tm		Tm	⊿Tm		Tm
T pTpTpTpTpTpTpTpT DNA (reference)		27.0 °C			20.3 °C			19.7 °C
UpUpUpUpUpUpUpUpU RNA (reference)		13.3 °C			16.7 °C			———
pc3UmpXpXpXpXpXpXpXpX modified oligo	X = T	27.4 °C	+ 0.4 °C	X = T	25.8 °C	+ 5.5 °C	X = T	25.5 °C
	X = U	16.5 °C	+ 3.2 °C	X = U	21.8 °C	+ 5.1 °C	X = U	21.0 °C
pUmpXpXpXpXpXpXpXpX c3-lacking oligo	X = T	27.4 °C	+ 0.4 °C	X = T	21.3 °C	+ 1.0 °C		———
	X = U	16.8 °C	+ 3.5 °C	X = U	21.8 °C	+ 5.1 °C		———
UmpUpUpUpUpUpUpUpU		———			20.2 °C	+ 3.5 °C		———
pUpUpUpUpUpUpUpUpU		———			20.6 °C	+ 3.9 °C		———

tions required for removal of the cyanoethyl groups and succinate linker, the cyclic structure was found to be stable.

The enzymatic digestion of pc3Um$(pT)_9$ with snake venom phosphodiesterase followed by alkaline phosphatase gave pc3Um and T in the expected ratio of 1:9, as evidenced by HPLC (Figure 3). Similarly, pc3Um$(pU)_9$ was also digested to give pc3Um$(pU)_9$ gave pc3Um and U in the 1:9 ratio.

The Thermal Stability of Duplexes of pUm$(pT)_9$ (or pUm$(pU)_9$) and Its Complementary d[A$(pA)_9$] (or A$(pA)_9$). The two synthetic oligonucleotides were tested for the thermal stability of the duplexes when bound to the complementary decadeoxyadenylate d[A$(pA)_9$] and decaadenylate A$(pA)_9$. The T_m values of four kinds of duplexes measured are summarized in Table 1. As the reference compounds, unmodified decathymidylate T$(pT)_9$ and decauridylate U$(pU)_9$ were used. In addition, two oligonucleotides, pUm$(pT)_9$ and pUm$(pU)_9$, were similarly synthesized on CPG gel to more exactly examine the effect of the cyclic structure on the thermal stability. The T_m values obtained from all possible duplexes between these acyclic oligonucleotides and d[A$(pA)_9$] or A$(pA)_9$ are listed in Table 1. The melting curves of all the duplexes tested are also shown in Figure 4. These results are discussed as follows by dividing the duplexes into four types, i.e., DNA*−DNA, RNA*−DNA, DNA*−RNA, and RNA*−RNA, where DNA* and RNA* refer to modified oligonucleotides pc3Um$(pT)_9$ and pc3Um$(pU)_9$, respectively, RNA refers to U$(pU)_9$ or A$(pA)_9$, and DNA refers to T$(pT)_9$ or d[A$(pA)_9$].

DNA*−DNA. Consequently, it was found that there is not significant difference in T_m among the duplexes between dA$(pA)_9$ and each of the oligothymidylates [Tp$(pT)_9$, pc3Um$(pT)_9$, and pUm$(pT)_9$], as shown in Figure 4A. The cyclic structure did not contribute to stabilization of the duplex since the duplex of pc3Um$(pT)_9$−dA$(pA)_9$ showed a melting curve profile similar to that of the duplex of pUm$(pT)_9$−dA$(pA)_9$, although these duplexes showed T_m values slightly higher by 0.4 °C than that of TpT_9−dA$(pA)_9$, as shown in Table 1.

RNA*−DNA. The thermal stability of pc3Um$(pU)_9$−dA$(pA)_9$ increased by 3.2 °C compared with that of U$(pU)_9$−dA$(pA)_9$ as shown in Figure 4B and Table 1. However, there is little difference (0.3 °C) in T_m between pc3Um$(pU)_9$−dA$(pA)_9$ (16.5 °C) and pUm$(pU)_9$−dA$(pA)_9$ (16.8 °C). Therefore, it was concluded that the enhancement of 3.2 °C is due to the presence of the 5′-phosphoryl and/or 2′-*O*-methyl groups.

RNA*−RNA. The thermal stability of pc3Um$(pU)_9$−A$(pA)_9$ increased significantly to the extent of 5.1 °C compared with that of U$(pU)_9$−A$(pA)_9$, as shown in Figure 4C and Table 1. However, almost the same melting curve profiles were obtained in both pc3Um$(pU)_9$−A$(pA)_9$ and pUm$(pU)_9$−A$(pA)_9$ which each has the T_m value of 21.8 °C. These results suggested that the 5′-terminal modification of RNA with this cyclic structure does not affect the thermal stability of a duplex with DNA or RNA. However, it is of interest that even pUm$(pU)_9$ having an acyclic structure enhanced considerably the binding ability with A$(pA)_9$ compared with the unmodified U$(pU)_9$. This unexpected finding suggests that RNA−RNA duplexes can be considerably stabilized by the 5′-terminal modification with both the 2′-*O*-methyl and 5′-terminal phosphate groups, or one of them. To clarify these phenomena, we also measured the T_m values of the duplexes, Um$(pU)_9$−A$(pA)_9$ and pU$(pU)_9$−A$(pA)_9$. These results are summarized in Table 1. These results are summarized in Table 1. It was concluded that each of the 5′-terminal phosphate group and the 2′−*O*−methyl group apparently contributes to duplex stabilization but not so much compared with the effect of pc3Um or pUm.

DNA*−RNA. A marked, striking effect of the propylene bridge of pc3Um$(pT)_9$ on the thermal stability of the duplex of pc3Um$(pT)_9$−A$(pA)_9$ was observed as shown in Figure 4D and Table 1. There is a clear-cut difference

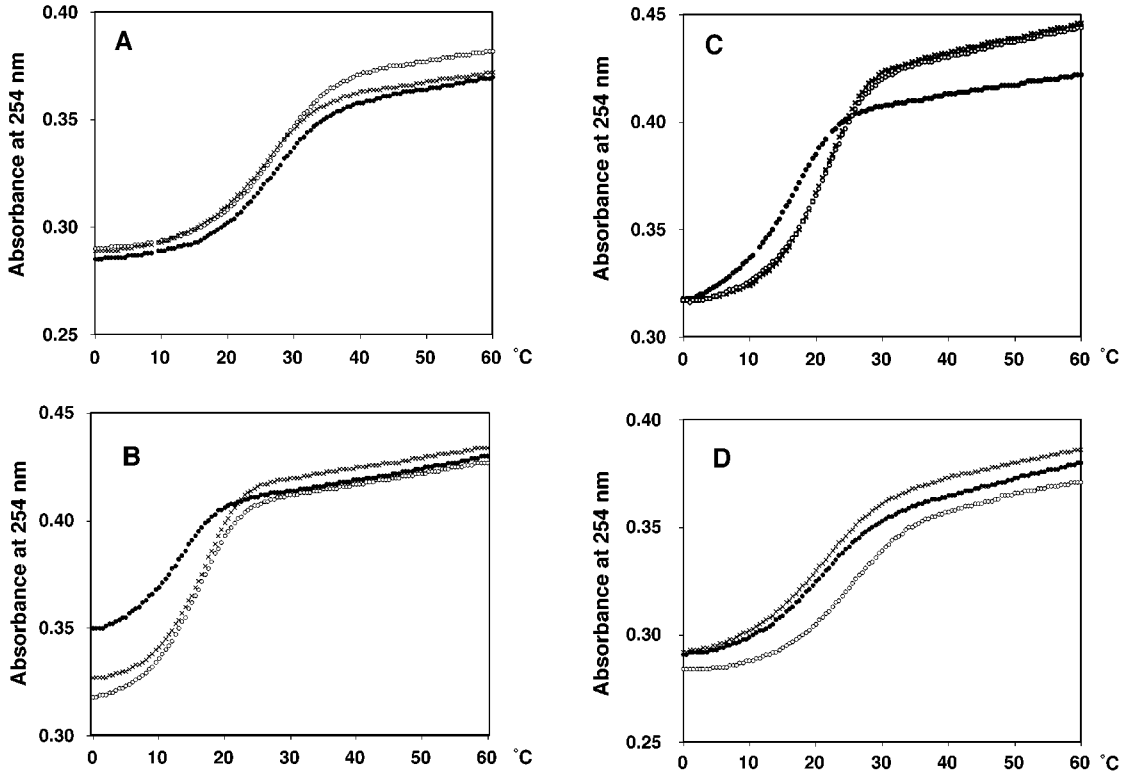


Figure 4. Melting curves of duplexes containing a cyclouridylic acid derivative (pC3Um), an acyclic uridylic derivative (pUm), and an unmodifed nucleoside (T or U). A: ● $T(T)_9-d[A(pA)_9]$, ○ $pc3Um(pT)_9-d[A(pA)_9]$, × $pUm(pT)_9-d[A(pA)_9]$; B: ● $U(pU)_9-d[A(pA)_9]$, ○ $pc3Um(pU)_9-d[A(pA)_9]$, × $pUm(pU)_9-d[A(pA)_9]$; C: ● $U(pU)_9-A(pA)_9$, ○ $pc3Um(pU)_9-A(pA)_9$, × $pUm(pU)_9-A(pA)_9$; D: ● $T(T)_9-d[A(pA)_9]$, ○ $pc3Um(pT)_9-A(pA)_9$, × $pUm(pT)_9-A(pA)_9$.

(5.5 °C) in T_m between $pc3Um(pT)_9-A(pA)_9$ and $T(pT)_9-A(pA)_9$, while the T_m value of $pUm(pT)_9-A(pA)_9$ is higher by 1.0 °C than that of $T(pT)_9-A(pA)_9$. The genuine contribution of the cyclic structure to the enhanced stabilization of $pc3Um(pT)_9-A(pA)_9$ is 4.5 °C. Such a remarkable change was not observed in the other cases, as described above. This marked difference is because the conformationally rigid A-type terminal uridine can form a more rigid, entropically favorable base-pair with the 5′-terminal inherent A-type adenosine of $A(pA)_9$. This effect is weaker in the duplexes DNA*−DNA and RNA*−DNA than that in the DNA*−RNA type duplex because the 5′-terminal thymidine of their complementary DNA strands has a more flexible property.

The T_m experiment of the duplex $pc3Um(pT)_9-A(pA)_8G$ with a mismatched G−U base-pair was also conducted. This result showed that this mismatched duplex has only a slightly decreased T_m value (ΔT_m = 0.3 °C) compared with that of $pc3Um(pT)_9-A(pA)_9$. The same tendency was also observed between $T(pT)_9-A(pA)_9$ and $T(pT)_9-A(pA)_8G$. The difference in T_m was only 0.6 °C. These results are in agreement with those recently reported by Turner,[28] who studied a variety of duplexes involving those mismatched only at the 3′-terminal position.

Conclusions

These results indicate that, when the cyclic structure is incorporated into the 5′-terminal site of oligodeoxynucleotides (DNA), it can enhance the binding affinity especially for RNA but not for DNA. This selectivity might be used for further designing antisense molecules which should target mRNAs.[3] Apart from this interesting result, we also found that, generally, the 5′-terminal modification of $U(pU)_9$ with pUm having both the 2′-*O*-methyl and 5′-terminal phosphate groups resulted in significant increase of binding affinity with their complementary DNA or RNA. Particularly, when the 5′-terminal U of $U(pU)_9$ was replaced with pUm, the stability of duplexes with $A(pA)_9$ was enhanced significantly with ΔT_m of 5.1 °C.

In duplexes, the 5′- or 3′-terminal site is apparently thermodynamically unstable and tends to be open faster than the middle site when heat is applied. Therefore, such a rigid cyclouridylic acid unit that can preserve the A-type whole conformation would work as a thermodynamically unchanged site to tightly keep the double strand with the complementary RNA.

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. UV spectra were recorded on a U-2000 spectrometer. 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, Waters). Reversed-phase HPLC was performed on an LC module 1 using a μBondasphere 5 μm C18

(28) Testa, S. M.; Disney, M. D.; Turner, D. H.; Kierzek, R. *Biochemistry* **1999**, *38*, 16655−16662.

100 Å (3.9 × 150 mm) column with a linear gradient starting from 0.1 M NH_4OAc, pH 7.0 and applying CH_3CN at a flow rate of 1.0 mL/min for 30 min at 50 °C. Ion-exchange HPLC was performed on a Gen-Pak FAX column (4.6 × 250 mm) with a linear gradient (10−67%) starting from 25 mM phosphate buffer (pH 6.0) and applying 25 mM phosphate 1 M sodium chloride buffer (pH 6.0) at a flow rate of 1.0 mL/min for 40 min at 50 °C. Pyridine was distilled two times from *p*-toluenesulfonyl chloride and from calcium hydride and then stored over molecular sieves 4A. Elemental analyses were performed by the Microanalytical Laboratory, Tokyo Institute of Technology at Nagatsuta.

3′-*O*-Acetyl-2′-*O*-methyluridine (5). 5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-methyluridine (**4**)[15] (6.2 g, 11.1 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (80 mL). To the mixture was added acetic anhydride (3.13 mL, 33.2 mmol), and stirring was continued at room temperature for 8 h. The reaction was quenched by addition of water, and the mixture was condensed to a half volume under reduced pressure. The mixture was partitioned between $CHCl_3$ (400 mL) and saturated aqueous $NaHCO_3$ (400 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was treated with 80% acetic acid (100 mL) at room temperature for 30 min, and the solvent was removed under reduced pressure. The residue was chromatographed on a column of silica gel (120 g) with $CHCl_3$−MeOH, 100:3, v/v) to give **5** as foam (2.94 g, 86%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 2.15 (3H, s), 3.34 (1H, br), 3.43 (3H, s), 3.79 (1H, d, $J_{5',5''}$ = 12.2 Hz), 3.95 (1H, d, $J_{5',5''}$ = 12.2 Hz), 4.19−4.23 (2H, m), 5.29 (1H, t, $J_{2',3''} = J_{3',4''}$ = 5.0 Hz), 5.76 (1H, d, $J_{5,6}$ = 8.3 Hz), 5.80 (1H, d, $J_{1',2''}$ = 5.0 Hz), 7.75 (1H, d, $J_{5,6}$ = 8.3 Hz), 9.51 (1H, br); ^{13}C NMR (68 MHz, $CDCl_3$) δ 20.76, 58.98, 61.40, 70.41, 81.21, 83.07, 89.60, 102.70, 141.46, 150.40, 163.45, 170.49. Anal. Calcd for $C_{12}H_{16}N_2O_7$: C, 48.00; H, 5.37; N, 9.33. Found: C, 47.69; H, 5.25; N, 9.23.

3′-*O*-Acetyl-5-iodo-2′-*O*-methyluridine (6). To a solution of 3′-*O*-acetyl-2′-*O*-methyluridine (**5**) (640 mg, 2.13 mmol) in dioxane (20 mL) was added ICl (0.45 mL, 3.94 mmol). The resulting mixture was stirred at room temperature for 2 h. The mixture was partitioned between $CHCl_3$ (150 mL) and 5% aqueous Na_2SO_3 (150 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (15 g) with $CHCl_3$−AcOEt, 70:30, v/v) to give **6** as foam (740 mg, 81%): ^{1}H NMR (270 MHz, C_5D_5N) δ 2.04 (3H, s), 3.48 (3H, s), 4.06 (1H, d, $J_{5',5''}$ = 11.9 Hz), 4.21 (1H, d, $J_{5',5''}$ = 12.2 Hz), 4.51 (1H, m), 4.66 (1H, t, $J_{1',2''} = J_{2',3''}$ = 5.0 Hz), 5.78 (1H, t, $J_{2',3''} = J_{3',4''}$ = 4.6 Hz), 6.61 (1H, d, $J_{1',2''}$ = 5.3 Hz), 9.21 (1H, s); ^{13}C NMR (68 MHz, C_5D_5N) δ 20.53, 58.61, 60.96, 71.55, 82.83, 84.05, 87.75, 145.27, 151.67, 161.44, 170.16. Anal. Calcd for $C_{12}H_{15}IN_2O_7$: C, 33.82; H, 3.55; N, 6.57; I, 29.78. Found: C, 34.18; H, 3.53; N, 6.56; I, 29.76.

3′-*O*-Acetyl-5-(2-hydroxypropynyl)-2′-*O*-methyluridine (7). A mixture of 3′-*O*-acetyl-5-iodo-2′-*O*-methyluridine (**6**) (4.27 g, 10.0 mmol) in DMF (100 mL) was degassed in vacuo and under irradiation with a supersonic wave apparatus by an aspirator and purged under argon. This procedure was repeated 5 times. To the mixture were added propyn-1-ol (1.75 mL, 30.1 mmol), triethylamine (2.79 mL, 20.0 mmol), CuI (381 mg, 2.0 mmol), and tetrakis(triphenylphosphine)palladium (1.16 g, 1.0 mmol). The resulting mixture was stirred at room temperature under argon for 1.5 h. After the mixture was evaporated under reduced pressure, the residue was coevaporated with xylene under reduced pressure. The mixture was chromatographed on a column of silica gel (200 g) with $CHCl_3$−AcOEt, 40−60, v/v) to give the crude product, which was dissolved in water−MeOH (1:1, v/v, 20 mL). The solution was passed through a column (150 mL) of Dowex 1 × 4 (CO_3^{2-} form). Washing was performed with water−MeOH (1:1, v/v). The solvent was removed under reduced pressure to give **7** (2.01 g, 57%): ^{1}H NMR (270 MHz, C_5D_5N) δ 2.05 (3H, s), 3.48 (3H, s), 4.05 (1H, d, $J_{5',5''}$ = 11.9 Hz), 4.19 (1H, d, $J_{5',5''}$ = 11.9 Hz), 4.49 (1H, m), 4.59−4.65 (3H, m, 2′−H), 5.78 (1H, t, $J_{2',3''} = J_{3',4''}$ = 4.5 Hz), 6.63 (1H, d, $J_{1',2''}$ = 5.4 Hz), 8.99 (1H, s); ^{13}C NMR (68 MHz, C_5D_5N) δ 20.62, 50.73, 58.73, 61.19, 71.67, 77.21, 82.81, 84.20, 87.65, 93.83, 100.67, 143.67, 151.11, 162.79, 170.25. Anal. Calcd for $C_{15}H_{18}N_2O_8 \cdot 0.4H_2O$: C, 49.83; H, 5.24; N, 7.75. Found: C, 49.61; H, 5.16; N, 7.41.

3′-*O*-Acetyl-5-(2-(hydroxypropyl)-2′-*O*-methyluridine (8). To a solution of 3′-*O*-acetyl-5-(2-hydroxypropynyl)-2′-*O*-methyluridine (**7**) (100 mg, 0.28 mmol) in dioxane (3 mL) was added Pd/C (30 mg) under hydrogen. The mixture was vigorously stirred at room temperature for 16 h. After being filtered by using Celite, the mixture was evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (2 g) with $CHCl_3$−MeOH (100:3, v/v) to give **8** as foam (92 mg, 91%): ^{1}H NMR (270 MHz, C_5D_5N) δ 2.05 (5H, s), 2.67 (2H, t, J = 7.4 Hz), 3.46 (3H, s), 3.82 (2H, t, J = 6.1 Hz), 4.06 (1H, d, $J_{5',5''}$ = 11.9 Hz), 4.19 (1H, d, $J_{5',5''}$ = 12.2 Hz), 4.48 (1H, m), 4.66 (1H, t, $J_{1',2''} = J_{2',3''}$ = 5.4 Hz), 5.81 (1H, t, $J_{2',3''} = J_{3',4''}$ = 4.3 Hz), 6.71 (1H, d, $J_{1',2''}$ = 5.6 Hz), 8.39 (1H, s), 13.3 (1H, s); ^{13}C NMR (68 MHz, C_5D_5N) δ 20.58, 24.02, 32.05, 58.63, 61.18, 61.48, 71.83, 82.42, 83.89, 87.38, 115.17, 136.40, 151.97, 164.66, 170.21. Anal. Calcd for $C_{15}H_{22}N_2O_8 \cdot 0.5H_2O$: C, 49.04; H, 6.31; N, 7.62. Found: C, 49.39; H, 6.18; N, 7.61.

Fully Protected Cyclouridylic Acid Derivative 10. A mixture of **8** (179 mg, 0.5 mmol) and 1*H*-tetrazole (168 mg, 2.4 mmol) was rendered anhydrous by repeated coevaporation with dry toluene and finally dissolved in acetonitrile−dioxane (25 mL-25 mL). To this mixture was added 2-(trimethylsilyl)-ethyl *N,N,N′,N′*-tetraisopropyl phosphorodiamidite[16] (209 mg, 0.6 mmol) dropwise over a period of 5 min. After the mixture containing the product **9** was stirred at room temperature for 30 min, *t*-BuOOH(0.5 mL, 5.0 mmol) was added. The resulting mixture was stirred for 20 min and then evaporated under reduced pressure. The residue was partitioned between $CHCl_3$ (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was dissolved in pyridine (5 mL), and concd ammonia (5 mL) was added. The resulting mixture was kept at room temperature for 3 h and then evaporated under reduced pressure. Extraction was performed with $CHCl_3$ (50 mL) and saturated aqueous $NaHCO_3$ (50 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (10 g) with $CHCl_3$−MeOH (100:1.5, v/v) to give **10a** as foam (81 mg, 34%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.0 (9H, s), 1.06−1.12 (2H, m), 1.73−1.83 (2H, m), 2.45−2.55 (2H, m), 3.63 (3H, s), 3.82 (1H, d, $J_{2',3''}$ = 5.3 Hz), 3.94−3.97 (2H, m), 3.98−4.07 (1H, m), 4.13−4.30 (4H, m), 4.66 (1H, d, $J_{5',5''}$ = 11.6 Hz), 5.92 (1H, s), 7.75 (1H, s), 9.71 (1H, s); ^{13}C NMR (68 MHz, $CDCl_3$) δ −1.58, 19.64, 19.71, 20.36, 26.96, 27.08, 58.55, 63.81, 64.75, 64.85, 66.96, 67.05, 67.58, 82.39, 82.52, 83.31, 86.94, 110.60, 137.74, 150.15, 163.65; ^{31}P NMR (109 MHz, $CDCl_3$, 85% H_3PO_4) δ −1.14. Anal. Calcd for $C_{18}H_{31}N_2O_9PSi \cdot 0.75H_2O$: C, 43.93; H, 6.66; N, 5.70. Found: C, 44.03; H, 6.43; N, 5.54. Further elution with $CDCl_3$−MeOH (100:2, v/v) gave **10b** as foam (84 mg, 35%): ^{1}H NMR (270 MHz, $CDCl_3$) δ 0.02 (9H, s), 1.07−1.13 (2H, m), 1.63−1.78 (1H, m), 1.89−2.03 (1H, m), 2.29 (1H, t, J = 12.0 Hz), 2.62−2.68 (1H, m), 3.25 (1H, br), 3.63 (3H, s), 3.83 (1H, d, $J_{2',3''}$ = 4.3 Hz), 3.91−4.41 (8H, m), 5.82 (1H, s), 8.00 (1H, s); ^{13}C NMR (68 MHz, $CDCl_3$, $CDCl_3$) δ −1.56, 19.55, 19.63, 20.88, 26.99, 58.51, 62.93, 65.02, 66.87, 67.55, 67.64, 81.98, 82.09, 83.45, 87.46, 109.88, 137.75, 150.08, 163.88; ^{31}P NMR (109 MHz, $CDCl_3$) δ −0.10. Anal. Calcd for $C_{18}H_{31}N_2O_9PSi \cdot H_2O$: C, 43.54; H, 6.70; N, 5.64. Found: C, 43.54; H, 6.38; N, 5.50.

Cyclouridylic Acid Derivative 3. Compound **10b** (39 mg, 82 μmol) was treated with 1 M TBAF·H_2O in THF (820 μL) at room temperature with stirring for 2 h. This solution was diluted with water and passed through a column of Dowex 50 W X 8 (pyridinium foam, 5 mL), and washing with water−pyridine (9:1, v/v) was performed. The eluate and washings were combined and evaporated under reduced pressure. The residue was dissolved in H_2O and charged on Whatman 3MM papers. Paper chromatography with *i*-PrOH−concd NH_3−H_2O (7:1:2, v/v/v) gave **3** (504 A_{260}, *Rf* 0.40, 62%): HPLC retention time 11.2 min; UV (H_2O) λ_{max} 268 nm, λ_{min} 235 nm; ^{1}H NMR

(400 MHz, D_2O) δ 1.65 (1H, m, J_{gem} = 14.7 Hz, J = 9.8 Hz, J = 3.3 Hz, $^4J_{H,P}$ = 3.1 Hz), 1.81 (1H, m, J_{gem} = 14.7 Hz, J = 7.2 Hz, J = 3.2 Hz), 2.46 (1H, m, J_{gem} = 14.5 Hz, J = 9.8 Hz, J = 3.2 Hz), 2.54 (1H, m, J_{gem} = 14.5 Hz, J = 7.2 Hz, J = 3.3 Hz,), 3.59 (3H, s), 3.83 (1H, m, J_{gem} = 10.1 Hz, $J_{H,P}$ = 4.4 Hz), 3.87 (1H, m, J_{gem} = 10.1 Hz, $J_{H,P}$ = 2.6 Hz), 4.09 (1H, dd, J = 4.9 Hz, J = 1.3 Hz), 4.12 (1H, dd, $J_{5',5''}$ = 11.8 Hz, $J_{5'',P}$ = 4.4 Hz, J = 1.5 Hz), 4.25 (1H, ddd, $J_{3',4''}$ = 8.1 Hz, $J_{4',5'}$ = 2.2 Hz, $J_{4',5''}$ = 4.4 Hz), 4.34 (1H, ddd, $J_{5',5''}$ = 11.8 Hz, $J_{5',P}$ = 2.2 Hz, $J_{4',5'}$ = 2.2 Hz), 4.45 (1H, dd, 3′−H, $J_{3',4''}$ = 8.1 Hz, $J_{2',3'}$ = 4.9 Hz), 5.97 (1H, d, $J_{1',2'}$ = 1.3 Hz, 1′-H), 8.06 (1H, s); ^{13}C NMR (68 MHz, D_2O) δ 22.89, 29.59 (d, J = 9.2 Hz), 60.93, 64.29, 64.51 (d, J 6.3 Hz), 69.74, 85.40 (d, J = 9.2 Hz), 86.03, 90.36, 113.62, 142.35, 154.03, 168.93; ^{31}P NMR (109 MHz, D_2O) δ 3.57; MS (FAB+) calcd for $C_{13}H_{20}N_2O_9P$ (M^+ − H) 379.0907. found 379.0916.

Cyclouridylic Acid 3′-Phosphoramidite Monomer Unit 11. Compound **10a** (78 mg, 0.162 mmol) was rendered anhydrous by repeated coevaporation with dry toluene and finally dissolved in dry CH_2Cl_2 (1.6 mL). To this mixture were added triethylamine (0.068 mL, 0.486 mol) and chloro(2-cyanoethoxy)(diisopropylamino)phosphine (58 mg, 0.243 mmol). The resulting mixture was stirred vigorously at room temperature for 1.5 h. The reaction was quenched by addition of ethanol (1 mL). The mixture was partitioned between $CHCl_3$ (10 mL) and saturated aqueous $NaHCO_3$ (10 mL). The organic layer was collected, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was chromatographed on a column of silica gel (5 g) with hexane−AcOEt−Et_3N, 50:50:1, v/v/v) to give **11** as foam (87 mg, 79%): ^{1}H NMR (270 MHz, $CDCl_3$) δ −0.06−0.07 (9H, m), 0.98−1.16 (14H, m), 1.60−1.80 (2H, m), 2.43−2.46 (4H, m), 3.42−4.17 (15H, m), 4.60−4.64 (1H, m) 5.87−5.89 (1H, m), 7.62−7.64 (1H, m; ^{13}C NMR (68 MHz, $CDCl_3$) δ −0.80, 19.50, 19.57, 20.02, 20.13, 22.59, 24.23, 24.37, 24.48, 26.81, 26.96, 42.90, 43.09, 44.94, 45.03, 57.61, 57.94, 58.01, 58.22, 63.29, 63.76, 64.51, 64.60, 66.54, 68.50, 68.72, 68.95, 69.15, 80.61, 82.57, 82.91, 87.69, 87.94, 110.21, 110.30, 117.22, 117.34, 137.22, 137.32, 150.42, 163.90; ^{31}P NMR (109 MHz, $CDCl_3$) δ −1.06, −0.06, 150.26, 150.98. Anal. Calcd for $C_{27}H_{48}N_4O_{10}P_2Si$: C, 47.78; H, 7.13; N, 8.25. Found: C, 48.00; H, 7.37; N, 8.11.

General Procedure for Solid-Phase Synthesis of Oligonucleotides Incorporating the Cyclouridylic Acid Unit. Chain elongation of a protected nonathymidylate or nonauridylate was performed on a DMTrT-loaded (44 μmol/g, 1 μmol) or DMTrU(TBDMS)-loaded (19.7 μmol/g, 1 μmol) CPG gel by the following standard protocol: (1) Detritylation with 3% trichloroacetic acid in CH_2Cl_2, (2) washing with pyridine followed by CH_3CN, (3) drying in vacuo, (4) condensation with an amidite unit (0.1 M, 20 equiv) in the presence of 1*H*-tetrazole (0.5 M, 100 equiv) in CH_3CN, (5) washing with pyridine, (6) capping with acetic anhydride−pyridine (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 in THF−pyridine−water (7:2:1, v/v/v) for 1 min. The average yield for condensation was estimated to be 99% by the DMTr cation assay. The solid support was placed in a glass column equipped with a glass filter, a stopper, and a cock. At the last stage, addition of the cyclouridylic acid monomer unit (14 mg, 20 μmol) to the growing chain of the nonamer on the CPG gel was done in CH_3CN (0.2 mL) in the presence of 1*H*-tetrazole (0.5 M, 100 equiv) for 10 min by using a glass vessel with a filter. The successive oxidation followed by washing with pyridine was performed using the same conditions as described above. Deprotection was performed as follows: The resin was treated with concd ammonia−pyridine (9:1, v/v, 2 mL) at room temperature for 20 h, and then the supernatant was collected by filtration and washing with pyridine was done. The solution and washings were combined and evaporated under reduced pressure. The residue was dissolved in 1 M TBAF·H_2O in THF (1 mL), and the solution was kept at room temperature for 16 h. The solution was charged on a column of Sepahdex G-15 (1.8 × 28 cm). Elution with water (20−50 mL) followed by lyophilization gave the crude product. Purification of this crude product by ion-exchange HPLC (FAX) followed by reversed-phase HPLC (C_{18}) gave pure c3Um(pT)$_9$ (23.1 A_{260}, 31%) or c3Um(pU)$_9$ (28.3 A_{260}, 31%).

General Procedure for Solid-Phase Synthesis of Oligonucleotides Incorporating 2′-*O*-Methyl-5′-uridylic Acid. The sequence of T(pT)$_8$ or U(pU)$_8$ on the solid support was synthesized according to the same procedure as described in the above experiment. The final condensation using 5′-*O*-DMTr-2′-*O*-methyluridine 3′-phosphoramidite unit was done in a manner similar to that described above. The 5′-terminal phosphorylation was performed by using 2-cyanoethyl [[2-(4,4′-dimethoxytrityloxy)ethoxy]sulfonyl]ethyl *N*,*N*-bis(diisopropyl) phosphoramidite[21] (0.2 M, 50 μmol) in CH_3CN (0.25 mL) in the presence of 1*H*-tetrazole at room temperature for 20 min. The successive oxidation was performed as described in the above experiment. Deprotection was done as follows: The CPG gel was evaporated under reduced pressure. The dried gel was treated with bis(trimethylsilyl)acetamide−pyridine (1:1, v/v, 0.4 mL) and gently rotated by using a rotary evaporator for 20 min. Then DBU (12 μL, 80 μmol) was added, and the flask was again rotated for an additional 10 min. The excess reagents and solvent were removed by filtration, and the resin was washed successively with pyridine and CH_2Cl_2. The resin was treated with 3% trichloroacetic acid in CH_2Cl_2 (1 mL × 4) and washed with CH_2Cl_2. A workup similar to that described in the above experiment gave pUmU$_9$ (38.0 A_{260}, 41%) or pUmT$_9$ (23.1 A_{260}, 30.6%). In a similar manner, pU$_{10}$ and UmU$_9$ were obtained and used for T_m experiments.

Enzyme Assay of Modified Oligonucleotides. A lyophilized oligonucleotide (1.0 A_{260}) was dissolved in 50 mM Tris-HCl, pH 8.0. The solution was incubated with snake venom phosphodiesterase (8 μL, 1 unit/μL) at 37 °C for 3 h. To the mixture was added alkaline phosphodiesterase {5 μL, (4 unit/μL)}, and the solution was incubated at 37 °C for 3 h. The mixture was analyzed by reversed-phase HPLC.

Acknowledgment. This work was partially supported by Toray Scientific Foundation and also supported by a Grant from "Research for the Future" Program of the Japan Society for the Promotion of Science (JSPS-RFTF97I00301) and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan. We thank Dr. Sei-ichiro Higashiya for his measurement of FAB mass.

Supporting Information Available: The ^{1}H, ^{13}C, and ^{31}P NMR spectra of the compounds **3**−**11**, UV spectra, and HPLC profiles of pc3Um(pU)$_9$, pc3Um(pT)$_9$, pUmU$_9$, and pUmT$_9$. This material is available free of charge via the Internet at http://pubs.acs.org.

JO991374H