Notes

Chemical Synthesis and Conformational Properties of a New Cyclouridylic Acid Having an Ethylene Bridge between the Uracil 5-Position and 5′-Phosphate Group

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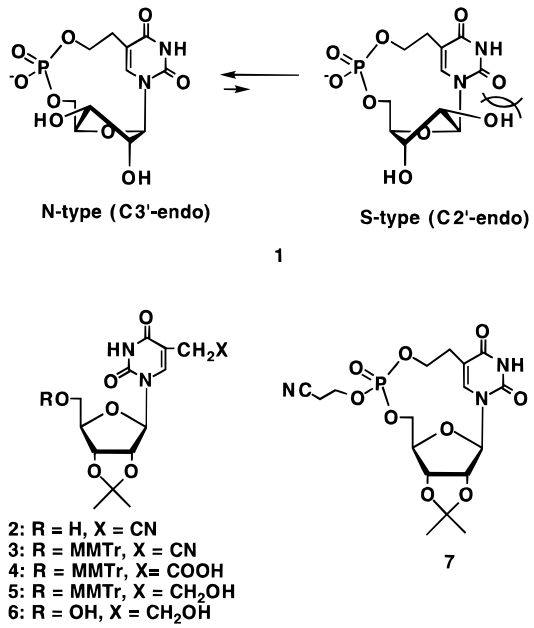
Introduction

Intramolecularly cyclized nucleosides and nucleotides have been utilized as useful analogues for studies of the conformational property or the stereochemistry of nucleic acids.[1] Chemical fixation of the glycosyl or 5′-*exo*-methylene torsion angle and the sugar conformation has been achieved by introducing various cyclic structures between the base and sugar moieties. However, most of the cyclonucleosides reported in the literature have conformations other than those of nucleotide units seen usually in DNA and RNA[2] because of their constrained structures. Therefore, it is of great importance to rationally design and synthesize cyclic nucleosides having fixed sugar conformations which resemble those of natural DNA and RNA.

A number of pyrimidine derivatives having an N-type sugar conformation stabilized by intramolecular interactions[3−6] have been discovered at the anticodon first letter of tRNAs, and their essential roles in codon recognition have been studied from a stereochemical point of view.[7] By use of NMR spectroscopy and molecular mechanics calculations, Sakamoto *et al.* investigated the conformational property of 5-[(methylamino)methyl]-uridine 5′-monophosphate (pmnm^{5}U), which was also found in the anticodon first letter of minor tRNA^{Arg} of *E. coli*,[8] and revealed that intrinsic intramolecular interactions between the 5′-phosphate and the 5-[(methylamino)-methyl] groups considerably stabilize the C3′-endo conformation.[5] This result suggests the possibility that the ribose moiety can be fixed in an N-type conformation by introducing a bridge between the 5′-phosphate group and the C-5 position of the uracil moiety.[9]

In this paper, we report the synthesis and conformational analysis of **1**, which was designed as an analogue of pmnm^{5}U cyclized intramolecularly via an ethylene bridge and expected to have a fixed N-type conformation.

Scheme 1



Results and Discussion

To synthesize **1**, 5-(cyanomethyl)-2′,3′-*O*-isopropylideneuridine (**2**)[10] was employed as the starting material (Scheme 1). Treatment of **2** with 1.5 equiv of 4-monomethoxytrityl chloride (MMTrCl) in pyridine gave the 5′-

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(1) (a) Maruyama, T.; Adachi, Y.; Honjo, M. *J. Org. Chem.* **1988**, *53*, 4552. (b) Yoshimura, Y.; Otter, B. A.; Ueda, T.; Matsuda, A. *Chem. Pharm. Bull.* **1992**, *40*, 1761 and references cited therein. (c) For a comprehensive review on cyclonucleosides, see: Mizuno, Y. *The Organic Chemistry of Nucleic Acid;* Elsevier: Amsterdam, 1986; pp 113−133.

(2) (a) Yamagata, Y.; Tomita, K.; Usui, H.; Sano, T.; Ueda, T. *Chem. Pharm. Bull.* **1989**, *37*, 1971. (b) Saenger, W. In *Principles of Nucleic Acid Structure*; Springer Verlag: New York, 1984. (c) Yamagata, Y.; Tomita, K.; Usui, H.; Matsuda, A.; Ueda, T. *Chem. Pharm. Bull.* **1992**, *40*, 6. (d) Bevierre, M. O.; Mesmaeker, A. D.; Wolf, R. M.; Freier, S. M. *Bioorg. Med. Chem. Lett.* **1994**, *2*, 237.

(3) (a) Egert, E.; Lindner, H. J.; Hillen, W.; Bohm, M. C. *J. Am. Chem. Soc.* **1980**, *102*, 3707. (b) Uhl, W.; Reiner, J.; Gassen, H. G. *Nucleic Acids Res.* **1983**, *11*, 1167.

(4) Kawai, G.; Hashizume, T.; Yasuda, M.; Miyazawa, T.; McCloskey, J. A.; Yokoyama, S. *Nucleosides Nucleotides* **1992**, *11*, 759. In this paper, the authors reported that *N*-acetyl-2′-*O*-methylcytidine (ac^{4}Cm) found in the anticodon first letter of various tRNA species has the preferred N-type conformer due to through-space and hydrogen bonding interactions.[3]

(5) Sakamoto, K. *et al.* To be published.

(6) For papers on other factors which control the equilibrium between an N- and an S-type conformer see: Uesugi, S.; Miki, H.; Ikehara, M.; Iwahashi, H.; Kyogoku, Y. *Tetrahedron Lett.* **1979**, 4073. Thibaudeau, C.; Plavec, J.; Garg, N.; Papchikhin, A.; Chattopadhyaya, J. *J. Am. Chem. Soc.* **1994**, *116*, 4038 and references cited therein.

(7) (a) Yokoyama, S.; Yamaizumi, Z.; Nishimura, S.; Miyazawa, T. *Nucl. Acids Res.* **1979**, *6*, 2611. (b) McCloskey, J. A.; Nishimura, S. *Acc. Chem. Res.* **1977**, *10*, 403. (c) Yokoyama, S.; Miyazawa, T. *J. Mol. Struc.* **1985**, *126*, 563. (d) Sierzputowska-Gracz, H.; Sochacka, E.; Malkiewicz, A.; Kuo, K.; Gehrke, C. W.; Agris, P. F. *J. Am. Chem. Soc.* **1987**, *109*, 7171. (e) Agris, P. S.; Sierzputowska-Gracz, H.; Smith, W.; Malkiewicz, A.; Sochacka, E.; Nawrot, B. *J. Am. Chem. Soc.* **1992**, *114*, 2652.

(8) Sakamoto, K.; Kawai, G.; Niimi, T.; Satoh, T.; Sekine, M.; Yamaizumi, Z.; Nishimura, S.; Miyazawa, T.; Yokoyama, S. *Eur. J. Biochem.* **1993**, *216*, 369.

(9) Maruyama *et al.*[1a] reported the synthesis of a cycloadenylic acid derivative bridged with a phosphorus atom between the adenine 8-position and the 5′-hydroxyl group. This compound exhibited the $J_{1',2'}$ value of 2.93 Hz and no coupling between 3′-H and 4′-H. This result suggests that this type of compound has an unusual sugar conformation.

(10) Ikeda, K.; Tanaka, S.; Mizuno, Y. *Chem. Pharm. Bull.* **1975**, *23*, 2958.

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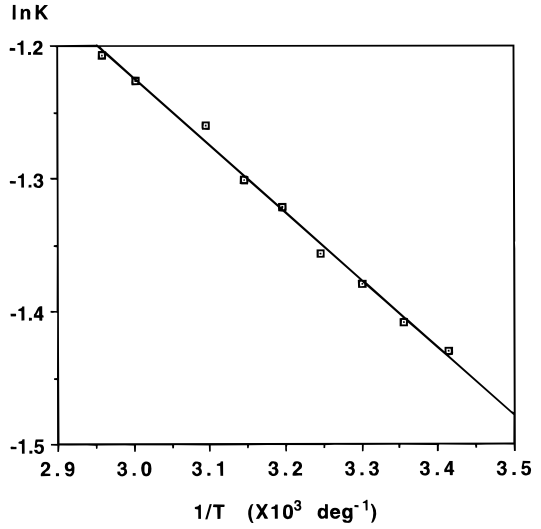

Figure 1. Temperature dependence of the equlibrium constants *K*[C2′-endo]/[C3′-endo] of **1**.

masked product **3** in 77% yield. The cyano group of **3** was hydrolyzed with 1 M NaOH−EtOH to give the carboxylic acid derivative **4** in 83% yield. Reduction of **4** with 1.7 equiv of borane−methyl sulfide complex[11] in THF gave the 5-(hydroxyethyl)uridine derivative **5** in 71% yield. Treatment of **5** with 1% trifluoroacetic acid in CH_2Cl_2 afforded **6** in 79% yield. Compound **6** was treated with 1.2 equiv of bis(diisopropylamino)(2-cyanoethoxy)phosphine in the presence of 1*H*-tetrazole. Successive oxidation using *tert*-butyl hydroperoxide gave the cyclized product **7** in 77% yield. Finally, the 12-membered ring phosphotriester **7** thus obtained was deprotected by treatment with concentrated ammonia−pyridine followed by hydrolysis with 60% HCOOH to give the desired product **1** in 80% yield. The structure of **1** was characterized by UV and ^{1}H, ^{13}C, and ^{31}P NMR.

In order to study the conformational property of **1**, all the $^3J_{H-H}$ values were determined. A significant conformational feature of **1** is the high population density of the N-type conformer as suggested by its very small $J_{1',2'}$ value (= 1.7 Hz). This *J* value is comparable to those of 5-nitrouridine ($J_{1',2'}$ = 1.7 Hz).[3a] Similar small $J_{1',2'}$ values were also reported in a series of naturally occurring 5-substituted 2-thiouridines.[7a,12] The fractional population of the N-type conformer in **1** was estimated to be 81% according to the equation of %N = $J_{3',4'}/(J_{1',2'} + J_{3',4'})$.[13]

To obtain more quantitative information about the sugar puckering of **1**, the thermodynamic parameters, ΔH and ΔS values, in the equilibrium between the N- and S-type conformers of **1** were calculated by the van't Hoff's plot analysis[15] (Figure 1) of the equilibrium constants *K* = % S/% N measured at different temperatures, which was obtained by NMR.

The ΔH and ΔS values were 1.0 kcal/mol and 0.6 cal/K·mol, respectively, showing a characteristic combination of relatively large positive ΔH and relatively small positive ΔS values compared to those of the other uridine nucleosides and nucleotides reported.[15] From these thermodynamic parameters, it was concluded that the N-type conformation of **1** is highly stabilized in an enthalpic manner due mainly to steric interaction between the 2-keto and 2′-hydroxyl groups[17] and is further stabilized in an entropic manner which arises from the cyclic structure.[18]

The 3D structure model of **1** was constructed by using MacroModel Version 4.5 (AMBER*)[19] with the aid of molecular mechanics and Monte Carlo algorithm by using the implicit treatment of solvent water with the GB/SA model.[20] In all simulations, the proton−proton distances estimated from the 1D-NOE and T1 values[21] and a dihedral angle (60°) around the C3′−C4′−C5′−O5′ bond from the ^{1}H-NMR *J* coupling data[22] were employed as the constraints. As shown in Figure 2, the two lowest energy structures (A, −957.26, and B, −958.76 kJ/mol) were found out of 1000 conformations. Both structures have the C3′-endo conformation with anti glycosyl angles (χ = −134° (A) and −162° (B)). These results are in agreement with those obtained by ^{1}H-NMR as mentioned above.

The CD spectrum of **1** in H_2O at 25 °C showed an enhanced positive Cotton effect ($[\theta]_{270\ nm}$ = 10 500) compared with that of uridine as shown in Figure 3. As seen in Figure 3, the CD spectrum of **1** at a high temperature of 80 °C was not affected essentially. These results also reflect the presence of a highly fixed anti-form.

The cyclic uridylic acid **1** having a phosphodiester linkage was completely resistant (24 h) to snake venom phosphodiesterase, nuclease P1, and calf spleen phosphodiesterase, which digest oligonucleotides to give 5′- or 3′-nucleotides. These results indicated that more flexible structures or dinucleotide units might be required for the enzymatic hydrolysis of internucleotidic phosphodiester linkages.

It is likely that there are at least three intrinsic factors which control the predominancy of N-conformers in modified uridines. One is the steric hindrance around

(11) Brown. H. C.; Choi. Y. M.; Narasimham, S. *J. Org. Chem.* **1982**, *47*, 3153.

(12) 5-Methoxy-2-thiouridine (mo^{5}s^{2}U, 1.7 Hz),[7d] 5-[(methoxycarbonyl)methoxy]-2-thiouridine (mcmo^{5}s^{2}U, 1.7 Hz),[7d] 5-[[(carboxymethyl)-amino]methyl]-2-thiouridine (cmnm^{5}s^{2}U, 1.8 Hz),[7d] 5-[(methoxycarbonyl)-methyl]-2-thiouridine (mcm^{5}s^{2}U, 2.3 Hz),[7a] 5-[(methylamino)methyl]-2-thiouridine (mnm^{5}s^{2}U, 2.3 Hz),[7a,d] 2-thiouridine (s^{2}U, 2.5 Hz).[7d]

(13) In general, the fractional population of the N- and S-conformers in a ribonucleoside can be estimated by several equations: (1) % N = $J_{3',4'}/(J_{1'2'} + J_{3'4'})$,[14] (2) % N = 1-($J_{1'2'}$/10) (if $J_{3'4'}$ can not be determined),[15] (3) % N = [(7.5 − $J_{1',2'}$)/6].[7d,16] To compare the relative rigidity of **1** with those of known modified uridine derivatives, we employed eq 1 in this study. The % N values of the above 5-substituted 2-thiouridines are given from the reported data[7a,d] of $J_{1'2'}$ and $J_{3'4'}$ as follows: mo^{5}s^{2}U, 82%; mcmo^{5}s^{2}U, 74%; cmnm^{5}s^{2}U, 77%; mcm^{5}s^{2}U, 78%; s^{2}mnm^{5}U, 78%; s^{2}U, 71%.

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

(15) Yokoyama, S.; Watanabe, T.; Murao, K.; Ishikura, H.; Yamaizumi, Z.; Nishimura, S.; Miyazawa, T. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4905.

(16) Sierzputowska-Gracz, H.; Guenther, R. H.; Agris, P. F.; Folkman, W.; Golankiewicz, B. *Magn. Reson. Chem.* **1991**, *29*, 885.

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

(18) Altona, C.; Sundaralingam, M. *J. Am. Chem. Soc.* **1973**, *95*, 2333.

(19) Mohamadi, F.; Richards, N. G.; Guida, W. C.; Liskamp, R.; Caufield, C.; Change, T.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

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

(21) (a) Rosevear, P. R.; Mildvan, A. S. *Methods Enzymol.* **1989**, *177*, 333. (b) Claude, R. J.; Sikakana, T.; Hehire, S.; Kuo, M. C.; Gibbsons, W. A. *Biophys. J.* **1978**, *24*, 815. (c) Kuo, M. C.; Gibbsons, W. A. *Ibid.* **1980**, *32*, 807.

(22) (a) Rousse, B.; Sund, C. Glemarec, C.; Sandstrom, A.; Agback, P.; Chattopadhyaya, J. *Tetrahedron* **1994**, *50*, 8711. (b) Rousse, B.; Puri, N.; Viswanadham, G.; Agback, P.; Glemarec, C.; Sandstrom, A.; Sund, C. Chattopadhyaya, J. *Ibid.* **1994**, *50*, 1777.

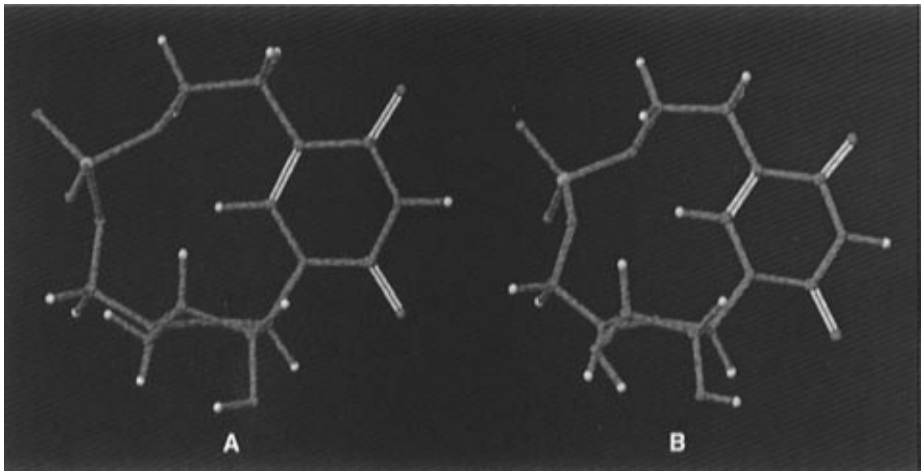

Figure 2. Two lowest energy conformations of **1**. Left (A): $E = -957.26$ kJ/mol. Right (B): $E = -958.76$ kJ/mol.

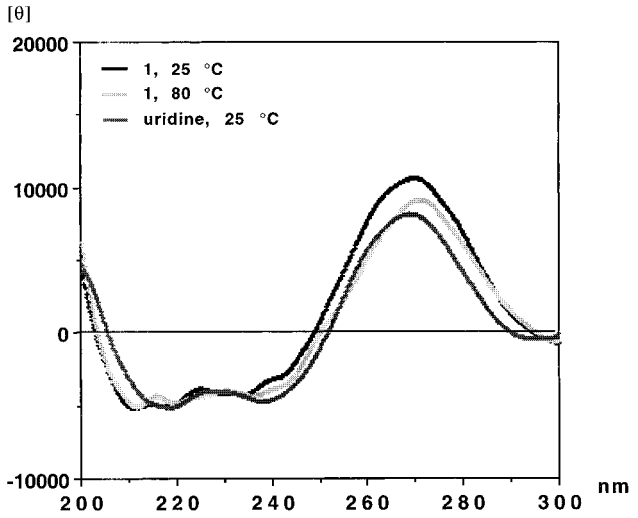

Figure 3. CD spectra of **1** and uridine in 10 mM phosphate buffer (pH 7.0) at 25 and 80 °C.

the 2′-hydroxyl group.[7a,17] It is well known that the rigid structure of s^2U is essentially attributable to the steric interaction between the bigger 2-(thiocarbonyl) group and the neighboring 2′-hydroxyl group.[7a,c,d] The second is the through-space interaction between the 4′-oxygen lone-paired electrons and the antibonding orbital of the 5-carbon of the 5,6 double bond of the uracil residue, as discussed in 5-nitorouridine.[3a,4] The third is the intramolecular interaction between the 5′-phosphate and the 5-substituent.[5] We observed that the cyclic covalent bonding bridge between the 5′-phosphate and 5-position of the uracil induces the 3′-endo conformation in **1**. This result implies that intramolecular interactions, such as hydrogen bonding and van der Waals contact, between the 5′-phosphate and the 5-[(methylamino)methyl] substituent are highly plausible as essential factors for stabilization of the 3′-endo form. Therefore, the compound **1** that we designed is rational for chemical fixation of the N-type conformation of uridylic acid.

It will be interesting to synthesize a cyclic U*pU derivative containing **1** (U* refers to a cyclic bridge structure) and to see if the cyclonucleotide **1** incorporated becomes more extremely rigid in the 3′-endo conformation, since Agris[23] reported that some 5-substituted 2-thiouridines are considerably stabilized in the N-conformation when incorporated into uridylate dimers. Further studies are needed to confirm this.

In conclusion, the present study suggests that the considerable predominance of the C3′ endo conformer in $pmnm^5U$ can be rationalized in terms of intramolecular hydrogen bond formation. Cyclic uridylic acid **1** should be useful as a thermodynamically rigid simple model of A-form nucleotides for conformational studies of nucleic acids.

Experimental Section

General Methods. TLC was performed by the use of Merck-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 choromatographic 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 a Waters LC module 1 using a μBondasphere C-18 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. Uridine was purchased from Yamasa Co., Ltd. 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.

5-(Cyanomethyl)-2′,3′-*O*-isopropylidene-5′-*O*-(4-monomethoxytrityl)uridine (3). Compound **2** (1.0 g, 3.1 mmol) was rendered anhydrous by repeated coevaporation with dry pyridine and finally dissolved in dry pyridine (30 mL). To the solution was added 4-monomethoxytrityl chloride (1.43 g, 4.6 mmol). After being stirred at room temperature for 15 h, the mixture was treated with water and then extracted with ether. The ethereal extract was washed three times with saturated $NaHCO_3$, dried over Na_2SO_4, filtered, and repeatedly coevaporated with toluene to dryness. The residue was dissolved in CH_2Cl_2 and chromatographed on a silica gel column with hexane−ethyl acetate (7:3, v/v) to give **3** (1.4 g, 77%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.38 and 1.60 (3H each, s), 2.77 (2H, br), 3.45−3.55 (2H, m), 3.80 (3H, s), 4.36 (1H, m), 4.82−4.89 (2H, m), 6.00 (1H, d, $J_{1',2'} = 3.0$ Hz), 6.85 (2H, d, $J = 8.9$ Hz), 7.26−7.41 (10H, m), 7.74 (1H, s); ^{13}C-NMR (68 MHz, $CDCl_3$) δ 15.01, 25.43, 27.24, 55.22, 63.61, 80.77, 84.62, 85.70, 87.14, 92.40, 105.18, 113.30, 114.54, 127.40, 128.03, 128.36, 128.43, 130.39, 134.52, 139.39,

(23) Agris *et al.* also reported that, in s^2UpU and s^2mnmUpU, s^2U and s^2mnmU exist essentially as the N-conformers: Smith, W. S.; Sierzutowska-Gracz, H.; Sochacka, E.; Malkiewicz, A.; Agris, P. F. *J. Am. Chem. Soc.* **1992**, *114*, 7989. On the other hand, it is also known that dihidrouridine is almost 100% S in the crystal (Emerson, J.; Sundaralingam, M. *Acta Crystallogr.* **1988**, *563*, 206) and in solution (Nawrot, B.; Malkiewicz, A.; Smith, W. S.; Sierzputoska-Gracz, H. Agris, P. F. *Nucleosides Nucleotides* **1995**, *14*, 143).

143.59, 158.83; FAB HRMS calcd for $C_{34}H_{32}O_7N_3$ $(M - H)^-$ 594.2241, found 594.2213.

5-(Carboxymethyl)-2′,3′-*O*-isopropylidene-5′-*O*-(4-monomethoxytrityl)uridine (4). Compound **3** (2.7 g, 4.4 mmol) was dissolved in 1 M NaOH−ethanol (50 mL−50 mL), and the mixture was stirred at 80 °C for 36 h. The solution was neutralized with Dowex 50W X 8 (pyridinium form, 20 mL), filtered, and evaporated to dryness. The residue was chromatographed on a silica gel column with CH_2Cl_2−MeOH (100:4−100:10, v/v) to give **4** (2.4 g, 83%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.38 and 1.60 (3H each, s), 2.77 (2H, br), 3.51 (2H, m), 3.77 (3H, s), 4.33 (1H, m), 4.88 (1H, m), 4.93 (1H, dd, $J_{1′,2′} = 2.3$ Hz, $J_{2′,3′} = 6.3$ Hz), 6.02 (1H, d, $J_{1′,2′} = 2.3$ Hz), 6.86−6.82 (2H, m), 7.21−7.42 (13H, m), 7.58 (1H, s, 6-H), 9.20, 9.84 (2H, br); ^{13}C-NMR (68 MHz, $CDCl_3$) δ 25.43, 27.24, 40.00, 55.15, 63.63, 65.77, 80.74, 84.42, 85.46, 86.96, 91.91, 108.88, 113.23, 114.41, 127.24, 127.92, 128.39, 130.33, 134.79, 139.60, 143.54, 143.68, 150.04, 158.71, 163.61, 174.29. Anal. Calcd for $C_{34}H_{34}N_2O_9 \cdot 3H_2O$: C, 61.63; H, 6.08; N, 4.23. Found: C, 61.52; H, 5.81; N, 4.17.

5-(Hydroxyethyl)-2′,3′-*O*-isopropylidene-5′-*O*-(4-monomethoxytrityl)uridine (5). Compound **4** (1.6 g, 2.6 mmol) was dissolved in dry THF (10 mL). A solution of borane−methyl sulfide (290 μL, 3.1 mmol) in THF (10 mL) was added dropwise over 30 min and stirred at room temperature. After the solution was stirred for an additional 1.5 h, borane−methyl sulfide (120 μL, 1.3 mmol) was added, and the mixture was stirred for 3.5 h. The solution was diluted with CH_2Cl_2 and washed three times with saturated $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2, and the CH_2Cl_2 extract was dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 and chromatographed on a silica gel column with CH_2Cl_2−MeOH (100:1.5, v/v) to give **5** (1.1 g, 71%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.30 and 1.53 (3H each, s), 2.06 (2H, m), 3.31−3.46 (6H, m), 3.73 (3H, s), 4.24 (1H, m), 4.76 (1H, dd, $J_{2′,3′} = 6.3$ Hz, $J_{3′,4′} = 3.3$ Hz), 4.80 (1H, dd, $J_{1′,2′} = 3.0$ Hz, $J_{2′,3′} = 6.3$ Hz), 5.93 (1H, d, $J_{1′,2′} = 3.0$ Hz), 6.78 (2H, d, $J = 8.9$ Hz), 7.18−7.26 (13H, m), 9.28 (1H, s); ^{13}C-NMR (68 MHz, $CDCl_3$) δ 25.43, 27.23, 30.32, 53.37, 55.17, 61.30, 63.58, 80.72, 84.15, 85.16, 86.83, 91.68, 112.69, 113.12, 114.50, 127.15, 127.85, 128.39, 128.43, 130.39, 134.79, 138.54, 143.74, 150.01, 158.67, 164.53. Anal. Calcd for $C_{34}H_{36}N_2O_8$: C, 67.99; H, 6.04; N, 4.66. Found: C, 67.53; H, 6.13; N, 4.74.

5-(Hydroxyethyl)-2′,3′-*O*-isopropylideneuridine (6). Compound **5** (1.0 g, 1.7 mmol) was dissolved in 1% trifluoroacetic acid−$CHCl_3$ (100 mL) and stirred for 2 h. The solution was partitioned between CH_2Cl_2−H_2O, and the organic layer was washed three times with saturated $NaHCO_3$, dried over Na_2SO_4, filtered, and repeatedly coevaporated with toluene to dryness. The residue was chromatographed on a silica gel column with CH_2Cl_2 to give **6** (432 mg, 79%): ^{1}H-NMR (270 MHz, $CDCl_3$−CD_3OD, 9:1, v/v) δ 1.36 and 1.58 (3H each, s), 2.52 (2H, t, $J = 5.6$ Hz), 3.69−3.77 (3H, m, 5″-H), 3.87 (1H, d, $J = 12.2$ Hz), 4.30 (1H, m), 4.88 (2H, m), 5.85 (1H, s), 7.59 (1H, s); ^{13}C-NMR (68 MHz, $CDCl_3$−CD_3OD, 9:1, v/v) δ 24.98, 26.90, 29.71, 60.04, 61.83, 80.32, 84.37, 86.58, 93.08, 111.27, 113.98, 139.05, 150.42, 164.37. Anal. Calcd for $C_{14}H_{20}N_2O_7$: C, 51.22; H, 6.14; N, 8.53. Found: C, 51.36; H, 6.43; N, 7.93.

Fully Protected Cyclonucleotide 7. Compound **6** (126 mg, 0.38 mmol) and 1*H*-tetrazole (106 mg, 0.46 mmol) were rendered anhydrous by repeated coevaporation with dry pyridine and successively with dry toluene and finally dissolved in CH_3CN (30 mL). Bis(diisopropylamino)(2-cyanoethoxy)phosphine (146 μL, 0.46 mmol) was added dropwise over 10 min. After the mixture was stirred for 30 min, *tert*-butyl hydroperoxide (380 μL, 3.8 mmol) was added, and the resulting mixture was stirred for an additional 1.5 h. The solvent was removed under reduced pressure, and the residue was partitioned between CH_2Cl_2−H_2O. The organic layer was washed three times with saturated $NaHCO_3$, dried over Na_2SO_4, filtered, and coevaporated repeatedly with toluene to dryness. The residue was chromatographed on a silica gel column with CH_2Cl_2−MeOH (100:1.5, v/v) to give **7** (169 mg, 77%).

One of the diastereoisomers: ^{1}H-NMR (270 MHz, $CDCl_3$−CD_3OD, 9:1, v/v) δ 1.28 and 1.48 (3H each, s), 2.40 (1H, m), 2.71 (2H, t, $J = 6.3$ Hz), 2.82 (1H, d, $J = 13$ Hz), 4.11−4.33 (6H, m), 4.66 (1H, br), 4.74 (1H, d, $J_{2′,3′} = 5.7$ Hz), 4.85 (1H, d, $J_{2′,3′} = 5.9$ Hz), 5.68 (1H, s), 7.42 (1H, s); ^{13}C-NMR (68 MHz, $CDCl_3$−CD_3OD, 9:1, v/v) δ 19.30 ($J_{C,P} = 7.5$ Hz), 26.24, 26.79, 29.42, 61.60 ($J_{C,P} = 4.8$ Hz), 68.40 ($J_{C,P} = 7.5$ Hz), 69.70 ($J_{C,P} = 7.5$ Hz), 80.67, 86.74, 95.42, 107.06, 113.01, 116.33, 137.84, 150.0, 163.67; ^{31}P-NMR (109 MHz, $CDCl_3$−CD_3OD, 9:1, v/v) δ −3.09.

One of the diastereoisomers: ^{1}H-NMR (270 MHz, $CDCl_3$−CD_3OD, 9:1, v/v) δ 1.36 and 1.51 (3H each, s), 2.63 (2H, t, $J = 5.6$ Hz), 3.07 (2H, br), 4.06−4.44 (7H, m), 4.84 (2H, m), 6.09 (1H, s), 7.46 (1H, s); ^{13}C-NMR (68 MHz, $CDCl_3$−CD_3OD, 9:1, v/v) δ 19.45 ($J_{C,P} = 7.5$ Hz), 24.85, 26.00, 26.54, 61.90 ($J_{C,P} = 4.8$ Hz), 67.60 ($J_{C,P} = 6.1$ Hz), 68.10 ($J_{C,P} = 7.5$ Hz), 79.73, 86.50 ($J_{C,P} = 8.8$ Hz), 91.52, 109.26, 113.93, 116.37, 137.32, 150.24, 163.56; ^{31}P-NMR (109 MHz, $CDCl_3$−CD_3OD) δ −3.12. Anal. Calcd for $C_{17}H_{22}N_3O_9P \cdot 1/2H_2O$: C, 45.14; H, 5.12; N, 9.29. Found: C, 45.56; H, 5.45; N, 8.29.

Cyclic Uridylic Acid Derivative 1. Compound **7** (220 mg, 0.5 mmol) was dissolved in concd ammonia−pyridine (25 mL−25 mL), and the resulting mixture was stirred for 1.5 h. The solvents were removed under reduced pressure, and the residue was dissolved in 60% HCOOH (50 mL). After 20 h, the solvent was removed and the residue was chromatographed on a C-18 reversed-phase silica gel column with water to give **1** (169 mg, 80%). The cyclonucleotide was further purified by reversed-phase HPLC: HPLC retention time, 3.6 min; UV λ_{max} 264 nm, λ_{min} 233 nm; ^{1}H-NMR (270 MHz, D_2O) δ 2.63 (2H, m), 4.19 (1H, ddd, $J_{4′,5″} = 2.4$ Hz, $J_{5′,5″} = 12.2$ Hz, $J_{5″,P} = 2.4$ Hz), 4.25−4.37 (3H, m), 4.40 (1H, dd, $J_{1′,2′} = 1.6$ Hz, $J_{2′,3′} = 4.5$ Hz), 4.46 (1H, dd, $J_{2′,3′} = 4.5$ Hz, $J_{3′,4′} = 6.9$ Hz), 4.50 (1H, ddd, $J_{4′,5′} = 2.4$ Hz, $J_{5′,5″} = 12.3$ Hz, $J_{5′,P} = 5.6$ Hz), 5.90 (1H, d, $J_{1′,2′} = 1.6$ Hz), 8.38 (1H, s); ^{13}C-NMR (68 MHz, D_2O) δ 22.79 ($J_{C,P} = 7.1$ Hz), 60.75 ($J_{C,P} = 6.1$ Hz), 61.34 ($J_{C,P} = 6.1$ Hz) 65.75, 72.52, 80.45 ($J_{C,P} = 8.6$ Hz), 87.87, 108.22, 134.32, 148.48, 163.59; ^{31}P-NMR (109 MHz, D_2O) δ 0.11; FAB HRMS calcd for $C_{11}H_{14}O_9N_2P$ $(M - H)^-$ 349.0437, found 349.0425.

Determination of Thermodynamic Parameters of the Ribose Puckering Using ^{1}H-NMR spectra. Compound **1** (50 A_{260}) was dissolved in 10 mM sodium phosphate buffer (700 μL), and the pH value was adjusted to 7.0 by addition of 1 M NaOH and lyophilized. The remaining H_2O was removed by repeated lyophylization three times with D_2O. The residue was finally dissolved in D_2O (700 μL). The vicinal coupling constants $J_{1′,2′}$, $J_{2′,3′}$, and $J_{3′,4′}$ at 20, 25, 30, 35, 40, 45, 50, 60, and 65 °C were measured quantitatively by using the Deconvolusion program implemented in the same spectrometer. The digital resolution after the Deconvolusion was 0.15 Hz. The fractional population of the S and N conformations, % S and % N, respectively, were obtained from the formula % N = $J_{3′,4′}/(J_{1′,2′}+J_{3′,4′})$ and % S = 1 − % N. The equilibrium constants, % S/% N, at each temperature were subjected to the van't Hoff's plot analysis with the aid of the method of least -squares analysis to give the enthalpy difference (ΔH) and the entropy difference (ΔS).

Table 1. T_1 Values of the Ribose Protons of Compound 1

proton	T_1 (s)
H6	1.7
H1′	3.4
H2′	1.9
H3′	1.5
H4′	0.9
H5′	0.4
H6′	0.5

***T*$_1$ and NOE Measurement.** Compound **1** (50A_{260}) was dissolved in 10 mM sodium phosphate buffer (700 μL), and the pH value was adjusted to 7.0 by addition of 1 M NaOH and lyophilized. The remaining H_2O was removed by repeated lyophilization three times with D_2O. The remaining oxgen gas was removed by repeated displacement of the air in the sample tube with argon. The T_1 values were measured according to the (selective saturation)−t_1−(observation) sequence where the t_1 values were set to 50−500 ms with the increment of 50 ms. The time dependence of the recovery ratio of saturated protons was quantified by comparison of the peak intensity with those of the nonirradiated peaks. The 1D-differential NOE spectra were measured using the noedif program implemented in the spectrometer where the presaturation time was set to 8 s. The quantity of the magnetization transfer to proton i from proton j, $\eta_i(j)$, were measured relative to the saturated peaks in the 1D-differential NOE spectra.

Determination of the Interproton Distances and the Conformation around the C4′−C5′ Bond. The cross relax-

Table 2. NOE Enhancement (%) of the Sugar Protons of Compound 1[a]

	H6	H1′	H2′	H3′	H4′	H5′	H5″
H6	*	3.4	4.9	9.1	0	0	0
H1′	1.2	*	6.0	0	1.4	0	0
H2′	5.8	14.5	*	−	0	0	−
H3′	8.8	2.8	−	*	0	0	1.6
H4′	3.5	2.9	0	0	*	0	1.3
H5′	−	−	−	−	−	*	−
H5″	1.2	0	0	3.0	4.5	34.4	*

[a] The first column represents the irradiated protons and the first row the observed protons. − indicates that the corresponding NOE was not observed quantitatively because of the nonselective irradiation of the neighboring proton signals.

Table 3. Cross-Relaxation Rates (σ) between Two Protons Calculated Using the NOE Enhancement Value (η) and the T_1 Value of Each Proton According to the Formula $\sigma_{ij} = \eta_i(j)/T_1{}^i$

	H6	H1′	H2′	H3′	H4′	H5′	H5″
H6	*	1.1	2.5	6.2	0	0	0
H1′	0.71	*	3.0	0	1.6	0	0
H2′	3.5	3.4	*	−	0	0	−
H3′	5.2	0.81	−	*	0	−	20.2
H4′	2.0	0.84	0	−	*	0	2.8
H5′	−	−	−	−	−	*	−
H5″	0.71	0	0	2.0	5.1	81.9	*

ation rate between protons i and j, $\sigma_{i,j}$, was estimated by the formula $\sigma_{i,j} = \eta_i(j)/T_1{}^i$. The interproton distance R_{ij} was estimated according to the formula $R_{i,j} = R_{5',5''}(\sigma_{5',5''}/\sigma_{i,j})^{1/6}$ [24] using $\sigma_{i,j}$ thus obtained and $R_{5',5''} = 1.8$ Å.[25] These results are summarized in Tables 1−4. The interproton distance between two protons, i and j, which was used in the computer simulation described below was the averaged value of $R_{i,j}$ and $R_{j,i}$ thus obtained.

The conformation around the C4′−C5′ bond was analyzed by using the equation % $g^+ = [13.3 - (J_{4',5'} + J_{4',5''})]/9.7$.[26] The % g^+ of **1** was calculated to be 89% at 25 °C.

3D Model Building of 1 Using Molecular Mechanics. The NMR refined structure of **1** was built using MacroModel Version 4.5 with the aid of molecular mechanics and Monte Carlo calculation using AMBER* force field.[19] The effect of solvation was included in these simulations by using the implicit treatment of solvent water with GB/SA model.[20] In all simulations, the proton−proton distances estimated from the 1D-NOE and T1 values[21] and a dihedral angle (60°) around the C3′−C4′−C5′−O5′ bond from the ^{1}H-NMR J coupling data[22] were employed as the constraints. A potential energy term representing the interproton distance restraints was added to the total energy of the system according to the formula $E = 0$ (if $(r - r_0)^2 < 0.04$ Å) or $E = k(r - r_0)^2$ (if $(r - r_0)^2 > 0.04$ Å),[27] where $k = 100$ kJ/mol and r_0 is the interproton distance derived from the NOE data described above. The torsion around the C4′−C5′ bond was also constrained to 60 ± 40° with the force constant of 1000 kJ/mol·rad^2 in order to reproduce the g^+ conformation which was suggested from the ^{1}H-NMR spectrum of **1** as described above. The initial structure was built from the uridine residue implemented in the MacroModel Version 4.5 structure file. In total, 1000 conformers were generated by using the MCMM option, and the structures were energy-minimized 500 times. Consequently, the two lowest energy structures (A, −957.26, and B, −958.76 kJ/mol) were obtained as shown in Figure 2. Both structures A and B have C3′-endo conformations with anti glycosyl angles χ (O4′−C1−N1−C2) = −134° and −162°, respectively.

Table 4. Interproton Distance (Å) Derived from the Cross-Relaxation Rates Listed in Table 3[a]

	H6	H1′	H2′	H3′	H4′	H5′	H5″
H6	*	3.7	3.2	2.8	−	−	−
H1′	4.0	*	3.1	−	3.5	−	−
H2′	3.1	3.1	*	−	−	−	−
H3′	2.8	3.9	−	*	−	−	2.3
H4′	3.3	3.9	−	−	*	−	2.3
H5′	−	−	−	−	−	*	−
H5″	4.0	−	−	3.3	2.9	1.8	*

[a] The distance between H5′ and H5″, 1.8 Å, was used as a reference. The cells where the correponding NOE were not observed or not quantitative are indicated with a −.

CD Spectra. The sample (0.47A_{260}) was dissolved in 10 mM sodium phosphate buffer (pH 7.0). The spectra were recorded at 25 and 80 °C in a 0.5 cm pathlength cell.

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Supporting Information Available: ^{1}H, ^{13}C, and ^{31}P NMR and mass spectra of the synthetic intermediates and the final product **1** (20 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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