Pergamon

Tetrahedron Letters, Vol. 36, No. 52, pp. 9515-9518, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)02062-4

Synthesis and Properties of Conformationally Rigid Cyclouridylic Acid Having a Covalent Bonding Linker between the Uracil 5-Position and the 5'-Phosphate Group

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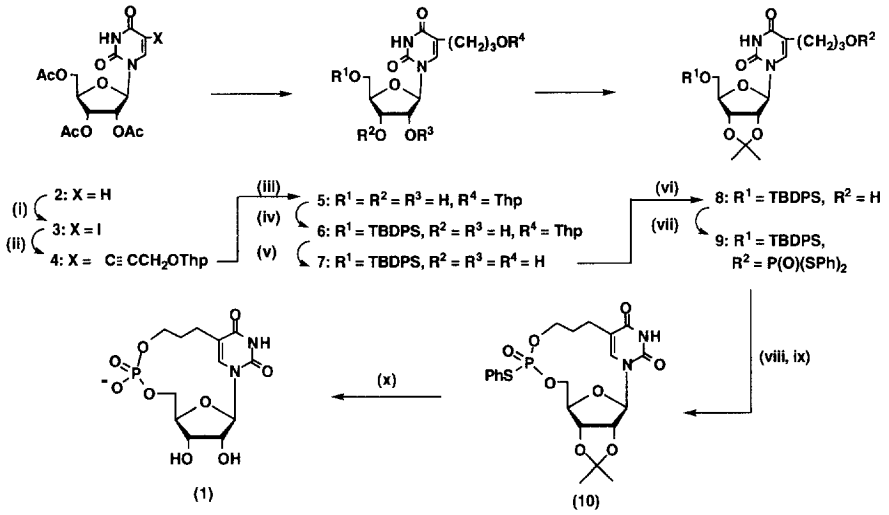
Abstract: A novel cyclouridylic acid **1** having a propylene bridge between the uracil 5 position and the 5'-phosphate group was synthesized. The structure of **1** was analyzed by ^{1}H NMR, and CD spectroscopy, which suggested that the cyclonucleotide **1** was highly stabilized in a considerably rigid g$^+$/C3'-endo conformation with the base orientation of anti. The molecular mechanics calculation of **1** gave a similar conclusion.

The antisense DNA/RNA strategy is one of the most straightforward ways to regulate gene expression and its medical applications have been widely studied by many groups.[1] In order to improve the efficiency of the antisense method, a number of new nucleic acid derivatives have been designed and synthesized. Most of such efforts have been focused on how to enhance the stability of the antisense DNA/RNA toward nucleases present in cells and its affinity for the target gene or mRNA by modifying the phosphate backbone or sugar moiety of natural nucleic acids.[1] A more sophisticated but less developed strategy is to incorporate cyclonucleosides having a conformation designed to stabilize duplexes. Most of the cyclonucleosides known to date, however, have unusual ribose or glycosyl conformations quite dissimilar to those seen in naturally occurring DNA or RNA duplexes because of their highly restrained structures;[2] Consequently, in general, they can not be used for the antisense strategy although several successful examples have been reported recently.[3] Therefore it is important to develop new cyclonucleosides having a rigid conformation which is similar to that of the nucleotide unit found in normal A-type or B-type duplexes. In our previous paper,[4] we reported that a cyclouridylic acid having an ethylene bridge between the uracil 5-position and the 5'-phosphate group has a predominant conformation of C3'-endo (81%)[5] over C2'-endo as suggested from its rather small $J_{1',2'}$ value (1.7 Hz).

Here we report the synthesis and properties of a new cyclonucleotide **1** having the hitherto smallest $J_{1',2'}$ value (1.1 Hz), which means an extremely high population (88%) of the C3'-endo conformer. This cyclic structure was designed on the basis of the previously discussed intramolecular interactions of the 5'-phosphate group of certain nucleotides with their base moieties: Sakamoto *et al.* suggested that 5-methyl-aminomethyluridine 5'-phosphate (pmnm^{5}U)[6a] found in the anticodon first letter of tRNA^{Arg} of *E. coli.* might exist predominantly in C3 endo conformation due to a hydrogen bonding interaction between the 5-substituent and the 5'-phosphate group.[6b] Poulter and Steiz also reported that pseudouridine (Ψ) in the anticodon stem of tRNA^{Phe} of *E. coli* or tRNA^{Gln} is stabilized as the C3'-endo conformer by a water-mediated hydrogen bond [NH$\cdots$O(H)-H$\cdots$$^-$O-P(O)] between HN (1) of Ψ and the 5'-phosphate group.[7] These papers imply that the

cyclic model **1** having a propylene bridge between the base and the 5'-phosphate fits more favorably the intramolecularly hydrogen-bonded structures of pmnm^{5}U and pψ as their covalent bonding mimics than the previous model[4] having the ethylene bridge.

The chemical synthesis of **1** is outlined in Scheme 1. 5-Iodouridine derivative **3**, obtained from 2', 3', 5'-tri-*O*-acetyluridine (**2**) by treatment with ICl,[8] was condensed with propargyl tetrahydropyranyl ether[9] by the Heck reaction[8] to give the 5-propinyl derivative **4** in 80% yield. The catalytic hydrogenation of **4** followed by alkaline hydrolysis gave the triol **5** in 65% yield. Treatment of **5** with *t*-butyldiphenylsilyl chloride gave the 5'-silylated derivative **6** in 99% yield. The Thp group of **6** was removed by the action of 80% acetic acid to give compound **7** in 69% yield. Compound **7** was converted to the 2', 3'-*O*-isopropylidene derivative **8** in 84% yield. This product was phosphorylated by using 1.2 equiv of cyclohexylammonium S, S-diphenyl phosphrodithioate (PSS), 1.2 equiv of isodurenedisulfonyl dichloride (DDS) and 4 equiv of 1*H*-tetrazole[10] to give the phosphorylated product **9** in 69% yield. One of the two phenylthio groups and the TBDPS groups of **9** were removed by successive treatments with bis(tributylstannyl) oxide and Bu_4NF and the resulting intermediate was treated with DDS and 1*H*-tetrazole to give the cyclized product **10** in 40% yield. Finally, **10** was deprotected by treatment with bis(tributylstannyl) oxide followed by 60% HCOOH to give **1** in 49% yield. The structure of **1** was confirmed by using ^{1}H, ^{13}C, and ^{31}P NMR and FAB mass spectroscopy.[11]



Scheme 1. Reagents and conditions: (i) ICl, CH_2Cl_2 reflux, 8 h (94%); (ii) propargyl tetrahydropyranyl ether, $Pd(PPh_3)_4$, CuI, triethylamine, DMF, 20 h (80%); (iii) Pd/C, H_2, EtOH, 25 h, then 0.5 M NaOH-pyridine, 10 min (65%); (iv) $PhB(OH)_2$, pyridine, 1 h, then *t*-butyldiphenylsilyl chloride, imidazole, DMF, 1.5 h (99%); (v) 80% AcOH, 12 h (69%); (vi) 2,2-dimethoxypropane, TMSCl, acetone, 20 h (84%); (vii) PSS, DDS, 1*H*-tetrazole, pyridine, 1 h (69%); (viii) $(Bu_3Sn)_2O$, pyridine, 1 h (quant) then $TBAF{\bullet}H_2O$, THF, 5h (quant) ; (ix) DDS, 1*H*-tetrazole, pyridine, 1 h (40%); (x) $(Bu_3Sn)_2O$, pyridine, 1.5 h then 60% HCOOH, 10 h (49%). All reactions were carried out at room temperature unless otherwise noted.

The ribose conformation of **1** was studied using 400 MHz ^{1}H-NMR. The ^{1}H-NMR spectrum of **1** showed a very small $J_{1'2'}$ value (1.1 Hz), indicating that the ribose pucker is highly fixed in the C3'-endo conformation which is found in the nucleotide unit in A type RNA. The other parameter defining the conformation of nucleotide is the χ angle which is well monitored by using circular dichroism spectroscopy.[2c, 12] The CD spectra of **1** at 25, 50 and 80 °C are shown in Fig. 1 along with that of uridine at 25°C. The CD spectrum of **1** at 25 °C, which is essentially identical with that of uridine with regard to the Cotton effect around 270, 240 and

220 nm, suggests that the glycosyl conformation of **1** is anti. At 80 °C, the intensity of the peak at 270 decreased only to a degree of 30% compared with that at 25 °C, suggesting that this compound is conformationally rigid as expected but the base orientation of **1** is a little flexible.

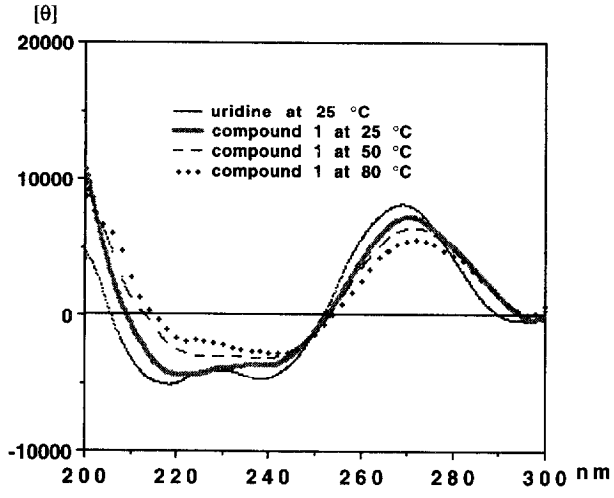
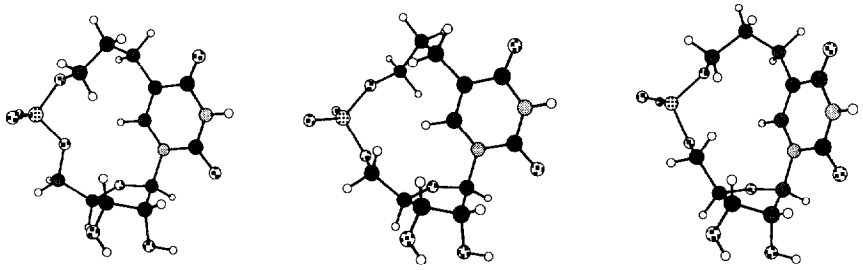


Figure 1. The CD Spectra of **1** at 25, 50, 80 °C and uridine at 25 °C in 10 mM phosphate buffer (pH 7.0).

In order to estimate the magnitude of fluttering of the χ angle, the molecular mechanics calculation was carried out by using Macro Model ver 4.5 program[13] with the AMBER* force field.[14] The effect of solvation was included in this calculation as implicit solvent water using GB/SA model.[15] The three lowest energy structures within 9 kJ/mol are shown in Fig. 2.[16] As shown in Fig. 2A, the overall conformation of the lowest energy structure is essentially identical with that of the nucleotide unit found in A type RNA having the C3'-endo sugar, anti glycoside (χ = -175.6 °) and g$^+$ conformation around the C4'-C5' bond (γ). Both the second and third lowest energy structures, shown in Figs. 2B and 2C, have C3'-endo sugar and anti glycoside conformations (χ = -174.2 and -162.3 °, respectively) similarly to those of the lowest energy conformer, except for the t conformation about γ which is not present in the usual A type RNA.



A: E = -574.24 kJ/mol **B**: E = -570.95 kJ/mol **C**: E = -569.71 kJ/mol

Figure 2. The low energy structures calculated using the AMBER* force field.

The χ angles of the three lowest energy structures are close to those of the nucleotide unit in A type duplexes, which vary from -146 to -166°.[2b] The energy difference (more than 3.29 kJ/mol) between the lowest energy structure and the second or third structure suggests the predominance of the structure depicted in Fig. 2A and this expectation is qualitatively in agreement with the calculated population of the g$^+$ conformation about γ (99 %) using the $J_{4',5'a}$ and $J_{4',5'b}$.[17] From these facts, we concluded that cyclonucleotide **1** exists mainly as the g+/C3'-endo conformer with the base orientation of anti as depicted in Fig. 2A, which is very similar to that of the nucleotide unit found in A type RNA duplex.

The present study provides a new strategy for the fixation of conformation of uridylic acid into that of the standard A type duplex of RNA. RNA or DNA oligomers containing such a phosphate-base bridged nucleotide unit are expected to have an enhanced affinity for their complementary strands and hence potential as antisense DNA/RNA molecules. Further studies on this methodology are in progress in our laboratory.

Acknowledgment. This work was supported by Ciba-Geigy Foundation for the Promotion of Science and a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture, Japan

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11. ^{1}H NMR (D_2O) δ 1.70 (2H, m, CCH_2C), 2.45 (2H, m, C5-CH_2), 3.84 (2h, m, C-CH_2-O), 4.10 (1H, dd, $J_{4',5'a}$ = 1.4 Hz, $J_{5'a,P}$ = 4.2 Hz, $J_{4',5'a}$ = 11.8 Hz, 5'-Ha), 4.27 (1H, m, 4'-H), 4.32 (1H, ddd, $J_{4',5'b}$ = 2.3 Hz, $J_{5'b,P}$ = 2.3 Hz, $J_{5'a,5'b}$ = 11.8 Hz, 5'-Hb), 4.38 (1H, dd, $J_{1',2'}$ = 1.1 Hz, $J_{2',3'}$ = 5.5 Hz, 2'-H), 4.38 (1H, dd, $J_{2',3'}$ = 5.5 Hz, $J_{3',4'}$ = 8.3 Hz, 3'-H), 5.86 (1H, d, $J_{1',2'}$ = 1.1 Hz, 1'-H), 8.02 (1H, s, 6-H); ^{31}P NMR (D_2O) 1.39 ppm; UV (H_2O) λmax 266.5 nm, λmin 203.5 nm. FAB HRMS 363.0593 calcd for $C_{12}H_{16}O_9N_2P$. Found 363.0584.
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(*Received in Japan* 22 *September* 1995; *revised* 23 *October* 1995; *accepted* 27 *October* 1995)