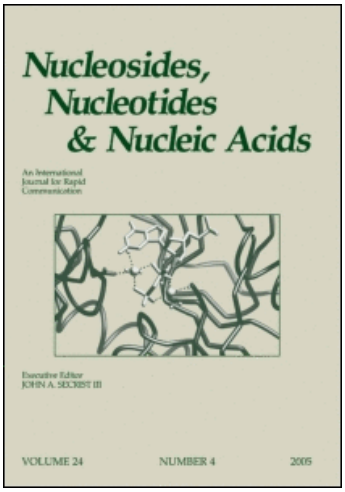

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Synthesis and Properties of Conformationally Rigid Cyclouridylic Acids Having Covalent Bonding Linkers Between the Uracil 5-Position and the 5'-Phosphate Group

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SYNTHESIS AND PROPERTIES OF CONFORMATIONALLY RIGID CYCLOURIDYLIC ACIDS HAVING COVALENT BONDING LINKERS BETWEEN THE URACIL 5-POSITION AND THE 5'-PHOSPHATE GROUP

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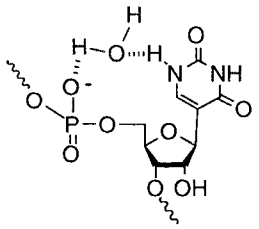
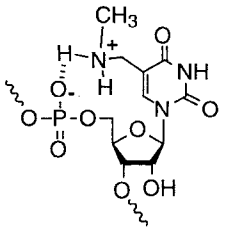
ABSTRACT Conformationally rigid cyclouridylic acid derivatives **2** and **3** having ethylene and propylene bridges, respectively, between the uracil 5-position and the 5'-phosphate group were synthesized. These intramolecularly cyclized compounds have predominantly the ribose pucker of C3'-endo and the g+ orientation around the C4'-C5' bond.

A number of modified nucleic acids have been synthesized to meet the criteria as new antisense oligonucleotides.[1] Fixation of the ribose pucker of antisense molecules in the C3'endo confomation, which is seen in the typical A-type RNA duplex, is favorable for hybridization with target mRNAs. Schultz and Gryaznov reported that oligonucleotides incorporating 3'-amino-2'-fluoro-2',3'-dideoxyribonucleosides showed high Tm values when hybridized with complementary RNAs.[2] Several research groups have studied antisense oligonucleotides containing nucleotide monomer building blocks with rigid cyclic structures.[3-7] Such modifications often caused unusual strains in the ribose ring so that the modified structures have unusual conformations.

To design fixation of the sugar pucker in the C3'-endo conformation rationally, we have searched for such rigid C3'-endo conformations from a pool of 3-dimentional structures found in naturally occuring tRNAs. Davis and Poulter have recently reported an interesting water-bridged cyclic hydrogen bonding in pseudouridine of tRNA.[8] This pseudouridine is also known to have the C3'-endo conformation. On the other hand, we have recently suggested that the C3'-endo confomation of 5-[(methylamino)methyl]-

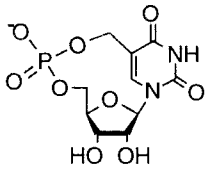


Pseudouridine

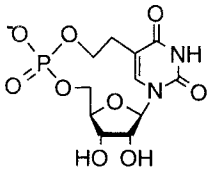


5-[(Methylamino)methyl]uridine

uridine found at the first letter of *E. coli* tRNA can be stabilized by the presense of an intramolecular hydrogen bond between the 5-substituent and the 5'-phosphate oxygen.[9] These facts led us to study the synthesis of intramolecularly cyclized UMP derivatives (**1**-**3**) having covalent bond linkers in place of the hydrogen bonding.



1



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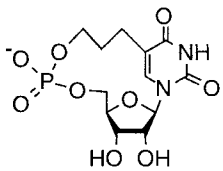
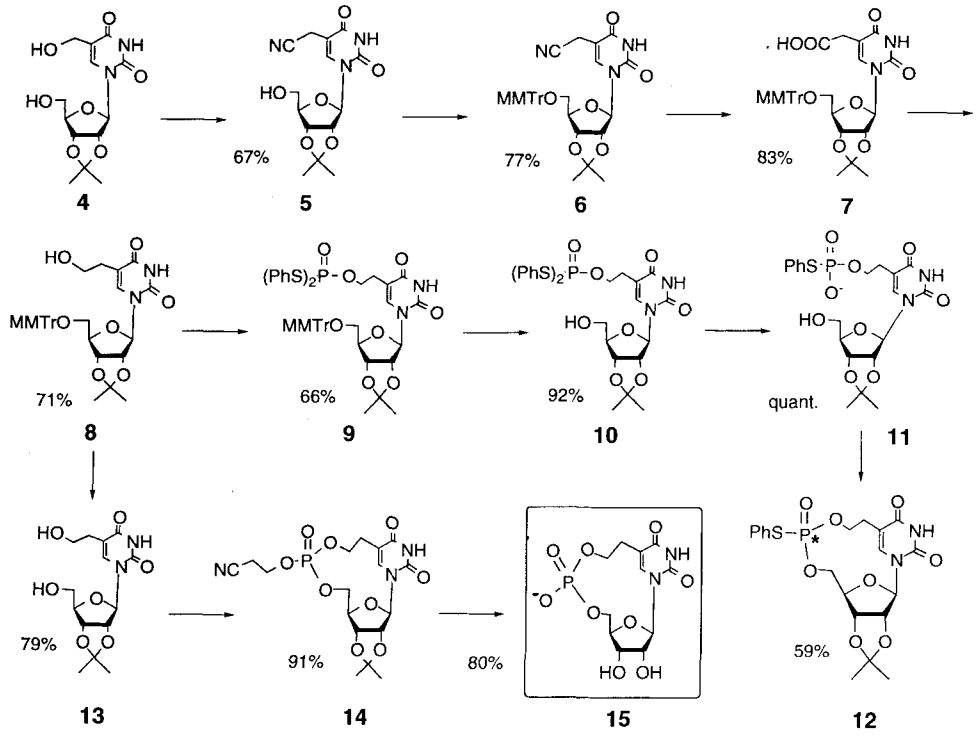


3

Synthesis of C2cUMP (2) and C3cUMP (3)

First, we tried to synthesize an intramolecularly cyclized UMP derivative **1** by various reactions. However, all attempts have failed to obtain **1**. Next, the synthesis of a cyclic UMP derivative **2** with an ethylene bridge was studied. This compoud was successfully synthesized as shown in Scheme 1.

2',3'-*O*-Isopropylidene-5-(hydroxylmethyl)uridine (**4**)[10] was treated with chlorotrimethylsilane in dioxane at 40 °C for 2.5 h, and reaction of the resulting 5-(chloromethyl)uridine derivative **5** with NaCN gave the 5-(cyanomethyl)uridine derivative **6** in 67% yield. Monomethoxytritylation of **6** followed by hydrolysis using 1 M NaOH-EtOH (1:1, v/v) at 80 °C for 18 h gave the 5-(carboxymethyl)uridine derivative **7** in 83% yield. Reduction of **7** with 1.7 equiv of $BH_3 \cdot S(CH_3)_2$ in THF at r.t. for 5 h gave the 5-(hydroxyethyl)uridine derivative **8** in 71% yield. To construct a cyclic structure between the 5-position of the uracil residue and the 5'-position *via* a phosphodiester linkage, we studied the cyclization *via* a stepwise phosphorylation: Phosphorylation of **8** with cyclohexylammonium *S,S*-diphenyl phosphorodithioate (PSS) in the presence of isodurenedisulfonyl dichloride (DDS)[11] and 1*H*-tetrazole gave the phosphorylated product **9** in 66% yield. Treatment of **9** with 1% trifluoroacetic acid in CH_2Cl_2 at r.t. for 30 min gave the 5'-hydroxyl compound **10** in 92 % yield. Treatment of **10** with $(Bu_3Sn)_2O$[12]



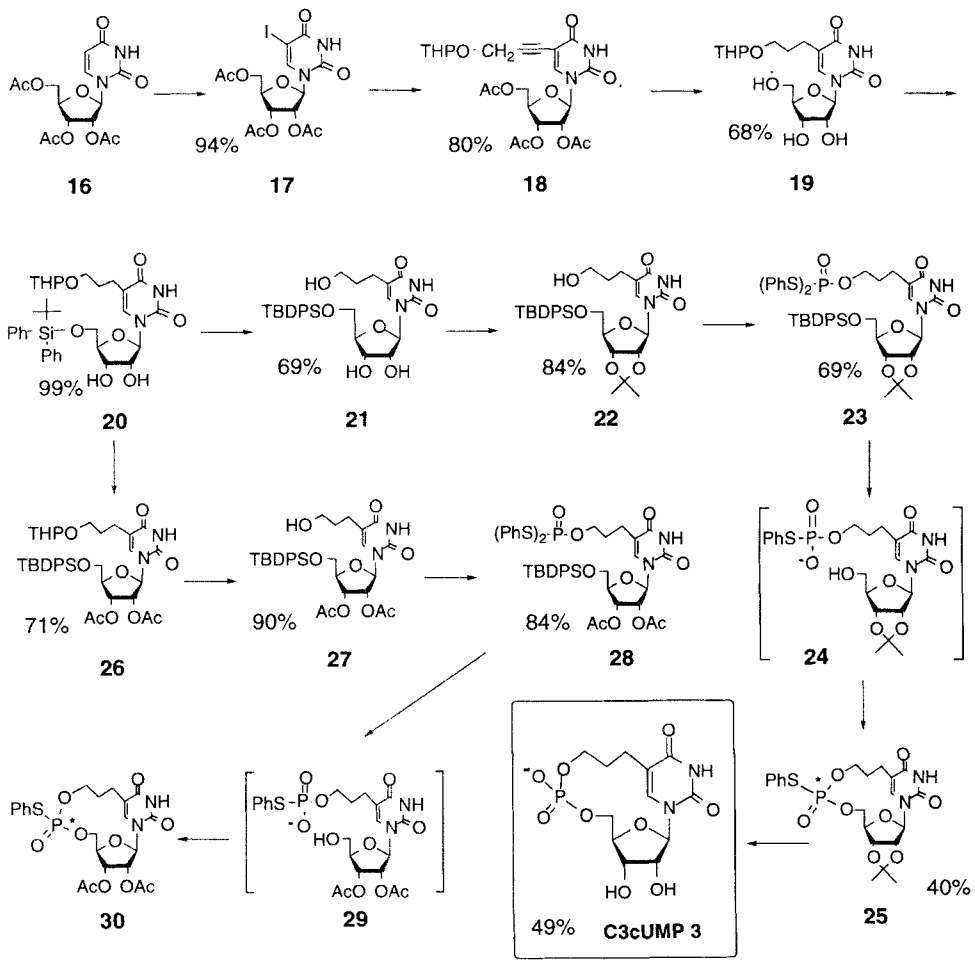
Scheme 1

in pyridine at r.t. for 3 h gave the *O,S*-phosphodiester **11** in quantitative yield. The final ring closure was carried out using DDS and 1*H*-tetrazole to give the desired cyclic triester **12** in 59 % yield. Interestingly, the ratio of the diasteromers is 9:1. The absolute configuration of these diasteromers was not determined.

To shorten the cyclization process, we also studied a more straightfoward method for the syntheis of C2cUMP **2**. Treatment of **8** with 1% trifluoroacetic acid in CH_2Cl_2 at r.t. for 2 h gave the diol derivative **13** in 79% yield. The phosphitylation of **13** with a bifunctional phosphitylating reagent $NCCH_2CH_2OP(NiPr_2)_2$ followed by oxidation with *t*-BuOOH gave the intramolecularly cyclized product **14** in 91% yield. The usual deprotection gave the desired product **2** in 80 % yield.

Synthesis of C3cUMP3

On the other hand, C3cUMP **3** was synthesized according to the following method. Reaction of 2',3',5'-tri-*O*-acetyluridine (**16**) with ICl[13] in CH_2Cl_2 under reflux for 2.5 h gave the 5-iodouridine derivative **17** in 94% yield. The successive Sonogashira-Robins



Scheme 2

reaction[14] resulted in formation of the 5-substituted derivative **18** in 80% yield. Hydrogenolysis of **18** followed by hydrolysis of the resulting intermediate gave the triol **19** in 68% yield. The selective 5'-*O*-silylation of **19** was carried out by use of TBDPSCl in the presence of phenylboric acid to give the 5'-silylated product (**20**) in quantitative yield. The diol **20** was further treated with 80% acetic acid to give the triol **21**. Reaction of **21** with 2 equiv of 2,2-dimethoxypropane in the presence of 0.1 equiv of chlorotrimethylsilane in acetone at r.t. for 20 h gave the 2',3'-*O*-isopropylideneuridine derivative **22** in 84% yield. Phosphorylation of **22** with *S*,*S*-diphenyl phosphorodithioate in the presence of DDS and 1*H*-tetrazole gave the phosphorylated

species **23** in 69% yield. Treatment of **23** with $(Bu_3Sn)_2O$ in pyridine followed by TBAF in THF gave the phosphorothioate derivative **24**, which was in turn treated with DDS and 1*H*-tetrazole to give the cyclic UMP derivative **25** in 40 % yield. Finally, C3cUMP **3** was obtained in 49% yield by treatment of **25** with $(Bu_3Sn)_2O$ followed by 60% HCOOH.

To study the effect of the protecting group of the 2'- and 3'-hydroxyl groups on the intramolecular cyclization, compound **20** was acetylated to give the diacetate **26** in 71% yield. The THP group was removed by treatment with 80% acetic acid to give the hydroxyl derivative **27** in 90% yield. This product was similarly phosphorylated to give compound **28** in 84% yield. A series of reactions similar to those as described above gave the cyclic UMP derivative **30** in 14% yield *via* the *O,S*-phosphodiester intermediate **29**. From these results, it was concluded that the isopropylidene group was superior to the acetyl group at the cyclization step.

Conformational Analysis of C2cUMP and C3cUMP

The CD spectra of C2cUMP and C3cUMP are shown in Figure 1. The change of these spectra at 25 and 80 °C are essentially similar to that of uridine. It seems that these bridge structures allowed flexibility of the orientation of the uracil base, although the degree of the changes in both cUMP derivatives is somewhat less than that of uridine.

The ribose puckers of C2cUMP and C3cUMP were analyzed on the basis of the H-H coupling constants according to the well known Altona equation.[15] The fractional populations of the C3'-endo confomer of C2cUMP and C3cUMP were calculated to be 81 and 88%, respectively. The g+ confomers of C2cUMP and C3cUMP around the C4'-C5' bond were also calculated to be 88 and 99%, respectively. As shown in these results, C3cUMP has a natural C3'-endo conformation judged from the sum (9.4 Hz) of $J_{1',2'}$ and $J_{3',4'}$, which is a normal value observed in the usual ribonucleosides.

Computer modeling of C2cUMP was constructed by calculation using the Amber* force field[16] of MacroModel ver 4.5[17] with GB/SA method[18] in consideration of constraints based on the NOE data. The lowest and second energy conformers of C2cUMP are shown in Figure 3.

The difference between the energies of the two conformers is only 0.36 kcal/mol. Therefore, C2cUMP might exist in equilibrium between these two comformers in aqueous solution. On the other hand, the sufficient NOE data could not be obtained in the case of C3cUMP because of the severe overlapping of proton signals. Therefore, the lowest energy confomation was estimated only by the MonteCaro method using Amber* force field of MacroModel ver 4.5. Consequently, the three lowest energy

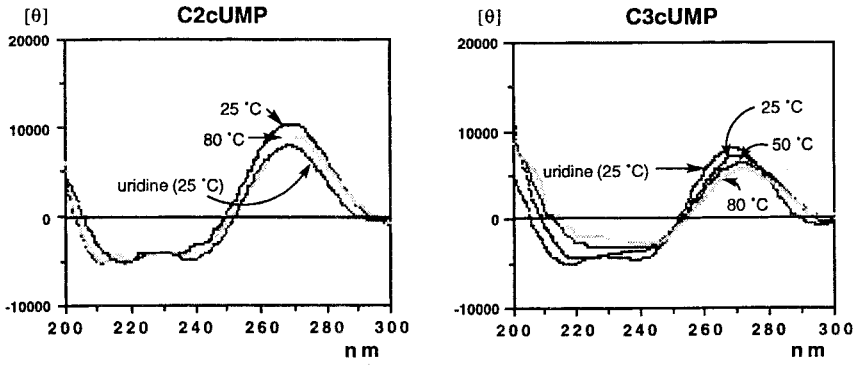


Figure 1. The CD spectra of C2cUMP **2** and C3cUMP **3** in 10 mM phosphate at pH 7.0

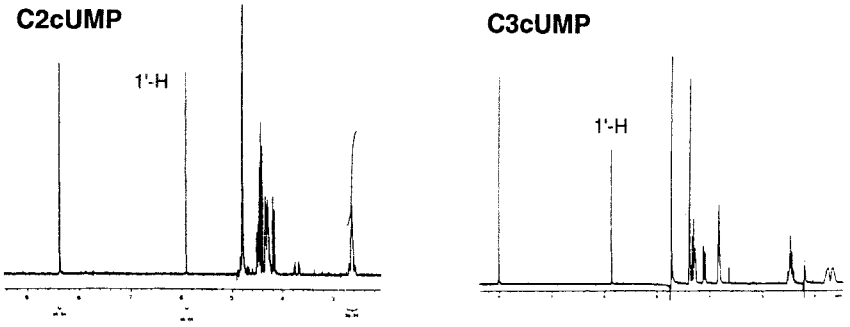


Figure 2. The ^{1}H NMR spectra of C2cUMP **2** and C3cUMP **3** in D_2O

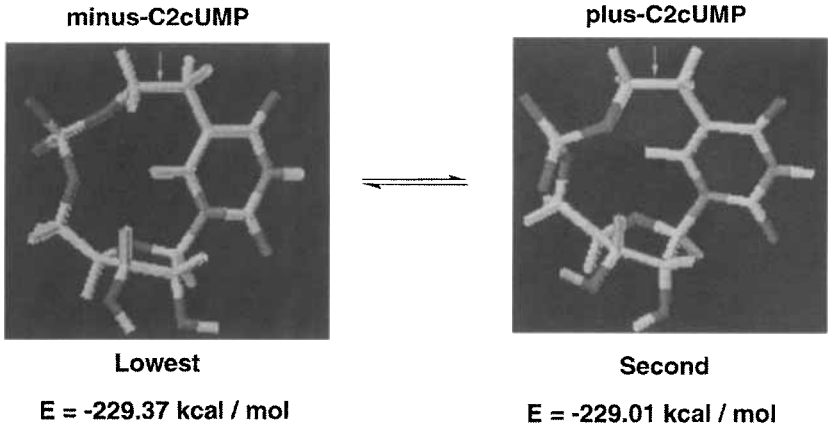
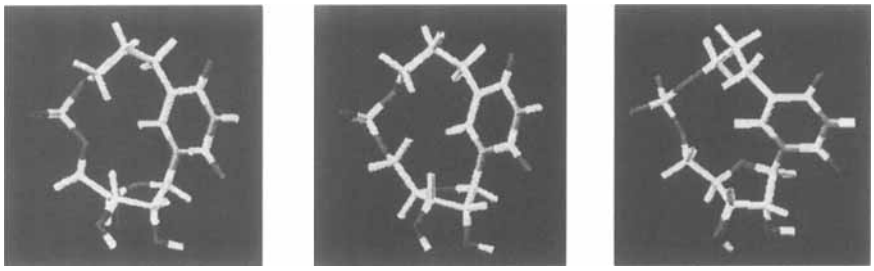


Figure 3. Two lowest energy conformations of C2cUMP calculated by the AMBER* force field of MacroModel ver 4.5 with GB/SA method based on Its NMR data



	Lowest	2nd	3rd	A type RNA
Energy (kcal / mol)	-137.38	-136.60	-136.29	—
Ribose pucker	C3'-endo	C2'-exo	C2'-exo	C3'-endo
γ	g^+	t	t	g^+
χ	-175.6	174.2	-162.3	-146 ~ -166

Figure 4. Two lowest energy conformations of C3cUMP calculated by the AMBER* force field of MacroModel ver 4.5 with GB/SA method

conformers were drawn as shown in Figure 4. The lowest energy conformer has the ribose pucker of C3'-end and the gauche plus (g+) torsion angle around the C4'-C5' bond, which are well consistent with those of A-type RNA. Both the second and third confomers have C2'-endo and *trans* conformations for the ribose pucker and the orientation around the C4'-C5' bond, respectively. However, the energy gap between the lowest and second or third confomers is 0.78 or 1.09 kcal/mol. Therefore, it is expected that C3cUMP might exsit mainly as the lowest confomer.

To estimate the variation of the proposed structures of C2cUMP and C3cUMP compared with a UpU/ApA dimer duplex, which was extracted from a typical A-type RNA duplex, these structures were superimposed with each other. The results are shown in Figurse 5 and 6. The base and ribose residues of both C2cUMP and C3cUMP are well imposed on the those of the 3'-downstream U of UpU/ApA dimer duplex fragment. In C2cUMP the 5'-phosphate did not match with that of with that of the 3'-downstream U of UpU/ApA, although the molecular dynamics simulation of C2cUMP suggested that there is a flexibility around the 5'-phosphate site. On the other hand, the 5'-phosphate group of C3cUMP can be put on the that of UpU well except for

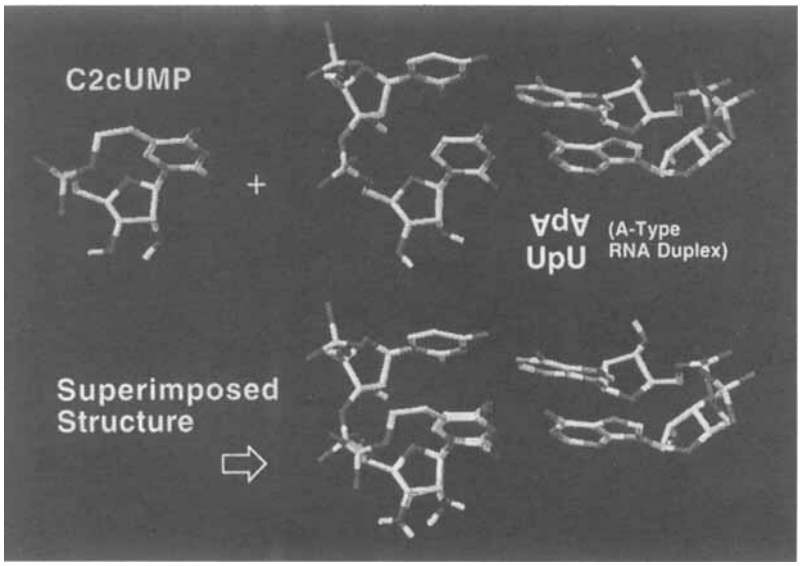


Figure 5. The superimposed structure of C2cUMP **2** on UpU/ApA dimer duplex which was extracted from A-type of RNA duplex

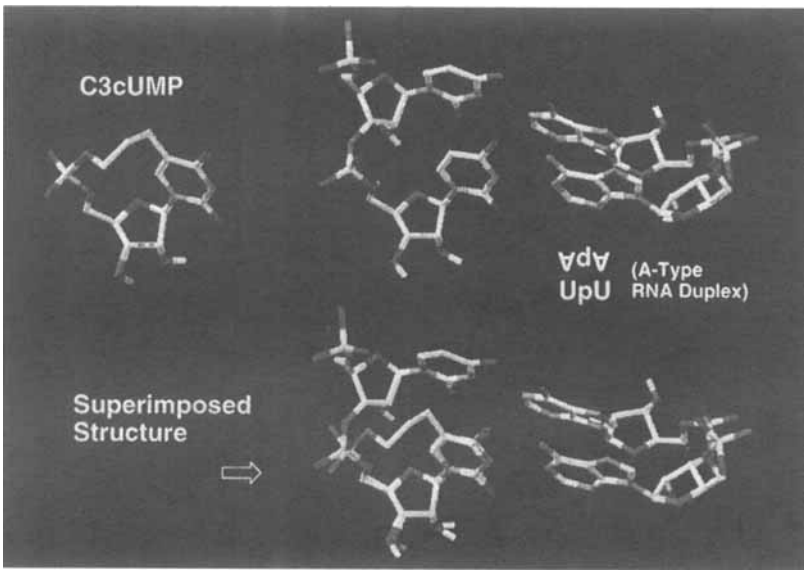


Figure 6. The superimposed structure of C3cUMP **3** on UpU/ApA dimer duplex which was extracted from A-type of RNA duplex

the direction of the P-O bond with a phosphate oxygen which can be attached to longer RNA chain. As judged from these superimposed structures, C3cUMP is expected to fit the A-type RNA duplex better than C2cUMP in a natural way. Based on these results, we are now studying incorporation of these cyclic structures into antisense RNAs.

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