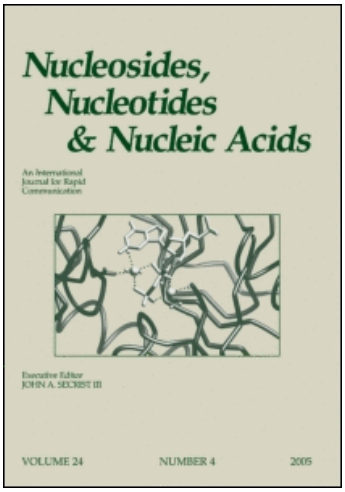

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SYNTHESIS OF ARTIFICIALLY BENT OLIGONUCLEOTIDES BY INCORPORATION OF CONFORMATIONALLY RIGID 5'-CYCLOURIDYLIC ACID DERIVATIVES

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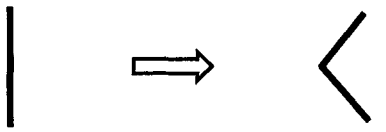
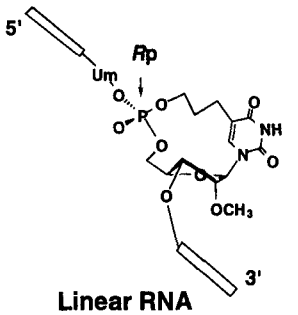
ABSTRACT: This paper describes the design and synthesis of a conformationally rigid dimer building block Ump*c*3Um having a propylene bridge linked between the uracil 5-position and 5'-phosphate group of pUm. Oligonucleotides incorporating the dimer unit with either the *S*p or *R*p configuration were synthesized by use of the phosphoramidite approach. The conformational properties of the dimer units and these oligonucleotides were studied in detail.

Bending of RNA is of great importance in molecular biology since functional RNA molecules such as tRNA or rRNA require such kinks to acquire active sites using 3D-holding. For example, the sharply bent U-turn structure at the anticodon loop is well known to make an important role in codon-anticodon recognition.[1] Recently, similar U-turn structures have been discovered at the active site of hammerhead ribozymes.[2] From the crystal structure of yeast $tRNA^{Phe}$,[3] we have also noticed many characteristic bent motifs with disordered structures of the base-orientation between proximal or consecutive nucleotide sequences. These unique structures are apparently essential for compact holding of the tRNA molecule. In other RNA loop regions, many bent structures have been found by many research groups.

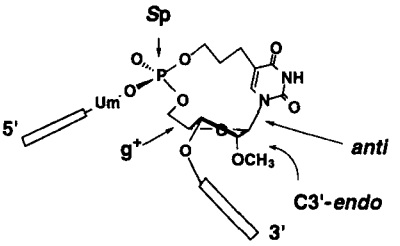


Linear DNA/RNA **Bent DNA/RNA**

Therefore, it is interesting if such a RNA bending structure can be artificially fixed by chemical methods, such studies would provide a new insight in functionalization of RNA as well as understanding of the structure-function relationship of RNA. To our best knowledge, however, no papers have been published about the synthesis of artificially bent oligonucleotides to date. Recently, much attention has been paid to incorporation of conformationally locked nucleosides into oligonucleotides in connection with the antisense strategy.[4] These modified oligonucleotides have been usually designed for enhancement of the binding affinity for the target mRNA or DNA, so that only nucleoside sugar moieties have been fixed.

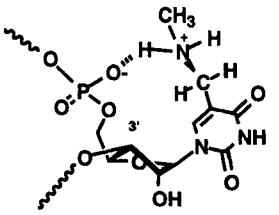


Linear RNA

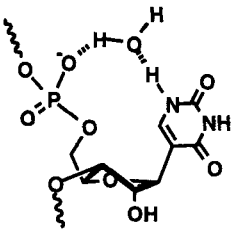


Bent RNA

In this paper, we report the first synthesis of bent oligonucleotides by use of a conformationally fixed dimer building unit having an artificially designed bent motif which has undoubtedly a rigid sugar conformation, an *anti* base-orientation, and a g^+ torsion angle around the P-$O^{5'}$ bond.



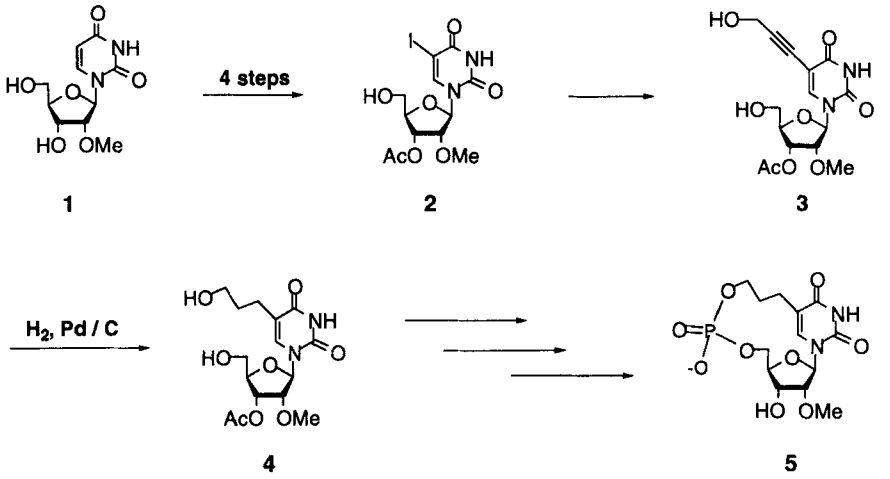
pmnm^{5}U



pΨ

We have quite recently studied the synthesis of conformationally fixed 5'-cyclouridylic acid derivatives.[5-7] Originally, these cyclic structures were designed as covalent-bonding mimics of conformationally rigid structural motifs, such as intramolecularly hydrogen-bonding 5-[(methylamino)methyl]uridine 5'-phosphate (pmnm^{5}U)[8] and water-bridged pseudouridine 5'-phosphate (pψ)[9] which are known to exhibit predominantly the C3'-*endo* conformation (A-type or N-type RNA) in the ribose moiety.

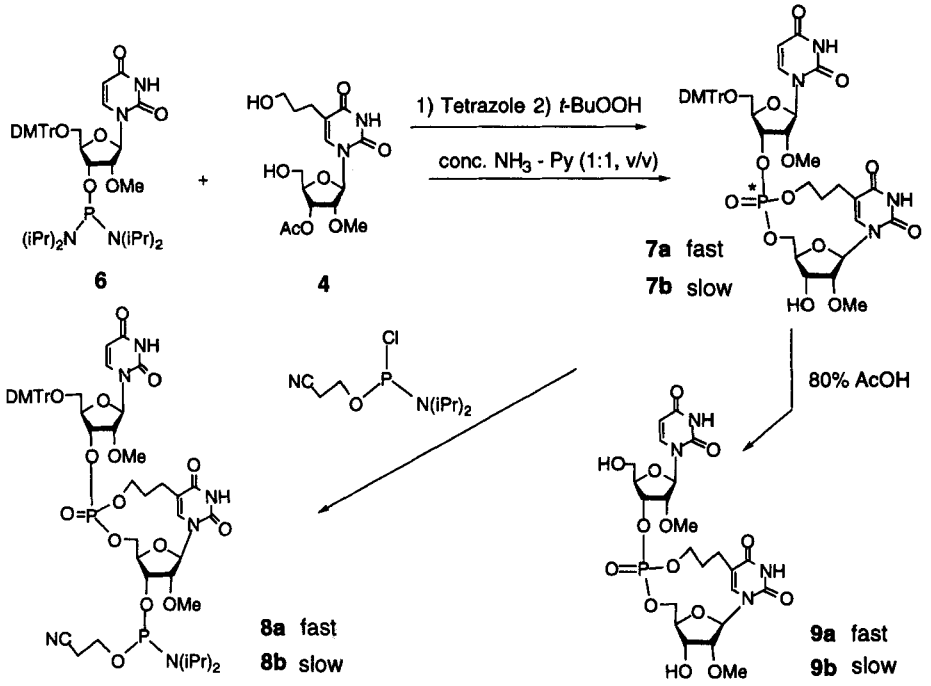
A 2'-*O*-methylcyclouridylic acid derivative **5** having a covalent-bonding propylene linker between the uracil residue and the 5'-phosphate group was designed and used as a bending motif in this study. The 2'-*O*-methyl group was introduced to simplify the chain elongation at the bending point. This compound was synthesized from 2'-*O*-methyluridine **1** *via* an 8-step reaction. First, compound **1** was converted to the 5-iodouridine derivative **2**. Compound **2** was further transformed to the 5-substituted product **3** according to the previously known method.[6] Further hydrogenation of **3** with H_2 on Pd/C gave the diol derivative **4** which was treated with a bifunctional phosphitylating reagent to give a phosphite triester which *in situ* was oxidized and then deprotected in the usual manner to give the final product **5**.



The detailed NMR analysis of **5** revealed that this cyclouridylic acid has a conformationally rocked structure similar to that of the typical A-type RNA duplex. Based on this finding we synthesized dimer phosphoramidite building blocks **8a,b** for incorporation of this bending motif into oligoribonucleotides. During the synthesis of **8a,b**, fortunately the two diastereomers of the synthetic intermediates DMTrUmpc3Um(fast) (**7a**) and DMTrUmpc3Um(slow) (**7b**) could be separated by silica gel column chromatography so that the pure diastereomers **8a** and **8b** were obtained from **7a** and **7b**, respectively. A diastereomeric mixture of **8a,b** was synthesized by simultaneous phosphitylation of **4** with the 2'-*O*-methyluridine 3'-(*N,N,N',N'*-tetraisopropyl)phosphorodiamidite derivative **6** in the presence of 1*H*-tetrazole. The unprotected dimers Umpc3Um(fast) **9a** and Umpc3Um(slow) **9b** were also synthesized from **7a** and **7b**, respectively, by detritylation.

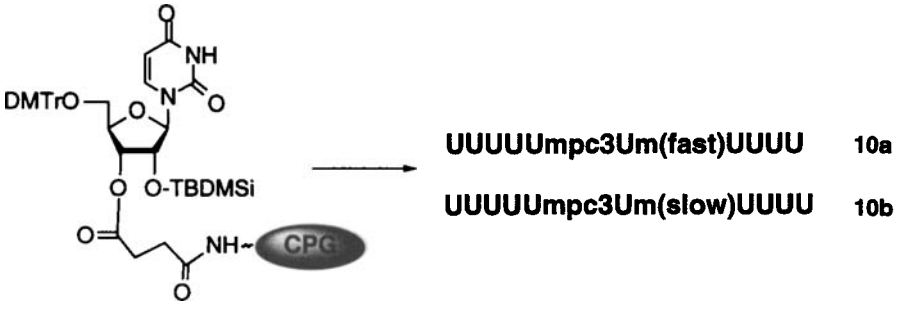
To examine the conformational properties of Umpc3Um(fast) **9a** and Umpc3Um(slow) **9b**, they were analyzed by NMR in detail. These NMR studies

disclosed that in both **9a** and **9b**, the upstream Um has preferentially a C2'-*endo* conformation and the downstream c3Um has a C3'-*endo* conformation. These conformational rigidity is important especially at the bending site.



By use of the phosphoramidite building blocks **8a** and **8b**, two decauridylates U_4Umpc3Um(fast)U_4 **10a** and U_4Umpc3Um(slow)U_4 **10b**, respectively, were synthesized by the standard protocol for automated synthesizers.

To determine the absolute configuration of Umpc3Um(fast) **9a** and Umpc3Um(slow) **9b** we calculated energy-minimized structures of **9a** and **9b** by fixing the ribose residues in the typical C2'-*endo* or C3'-*endo* conformation by the Monte Calro method using MacroModel ver 5.0. These results indicated the modified decauridylate having the *R*p configuration at the cyclouridylic acid should have a higher hybridization ability than that having the *S*p configuration.



Based on this assumption, the Tm experiments of duplexes formed between these modified decauridylates and their complementary decaadenylate were conducted. As the result, it was found that the decauridylate having U_4Umpc3Um(slow) exhibited a higher Tm value than that having U_4Umpc3Um(fast). From these results, it was strongly suggested that U_4Umpc3Um(fast) should have the *Sp* configuration, which enabled us to create bending oligoribonucleotides.

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