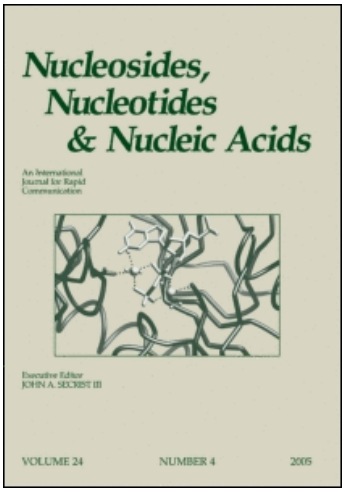

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Oligonucleotides Containing a 6-Substituted Pyrimidine Base: A Design for Myb Inhibitors

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OLIGONUCLEOTIDES CONTAINING A 6-SUBSTITUTED PYRIMIDINE BASE: A DESIGN FOR MYB INHIBITORS

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ABSTRACT: An oligonucleotide having a 6-formylpyrimidine nucleoside in the Myb binding sequence was synthesized based on computer calculation to fit the DNA-protein binding structure.

The incorporation of 2'-deoxy-5-substituted uridine derivatives into oligonucleotides (ONs) is a common design target for the study on stability of the duplex and analysis of the DNA structure and characters. However, ONs bearing 6-functionalized pyrimidine nucleosides have not been thoroughly studied.

We were interested in investigating whether the 6-formylpyrimidine base containing ONs could form a stable duplex, and if possible, whether a DNA binding protein, for example the proto-oncogene product Myb, could contact the modified protein-binding sequence, in which the 6-formyl group would be able to interact with proximal basic amino acid residues in the ON-protein complex. Computer calculation, based on the 3-D structure of a specific DNA complex of the Myb DNA-binding domain,[1)] showed that only one particular position could be possible for replacement of thymine base by 6-formylpyrimidine to set a short distance between the 6-formyl group and the basic amino acid residue, which was the guanidino group of Arg-190.

The precursor of the 6-formyl pyrimidine nucleoside, 1-[2'-(*O*-methyl)ribofuranosyl]-6-(1,2-diacetoxy)ethyl-4-ethoxy-2-pyrimidinone (Py*),[2)] appropriate for a DNA synthesizer, was synthesized in a gram scale from O^2,2'-cyclouridine as the following sequence: 1) 2'-*O*-methylation by the ISIS

method (89%);[3] 2) change of the base structure to 4-ethoxy-2-pyrimidinone (83%) for effective C6-lithiation and iodination (87%), 3) Stille coupling with tributyl(vinyl)tin (90%), 4) dihydroxylation of the resulting vinyl group using OsO_4 (84%), and 5) acetylation of the vicinal diol and selective deprotection of 3',5'-dihydroxyl groups (78%). Dimethoxytritylation of the 5'-hydroxyl group followed by 3'-*O*-phosphitylation afforded the nucleoside phosphoramidite unit ready to incorporate into the 23-mer including the Myb binding sequence, *i.e.* 3'-TGTGGGATPy*GACTGTGTGTAAGA-5'.[4] After ammonolysis, the 23-mer was purified with HPLC and treated with $NaIO_4$ to create the 6-formyl at the Py* position. Enzymatic hydrolysis and HPLC analysis proved the existence of 6-formyl-2'-*O*-methylcytidine in the strand.[5]

Stability of the duplex with its almost complementary strand decreased (ΔTm -5 °C), and the computer modeling displayed the structure of the duplex was somewhat between A- and B-forms of DNA. Gel mobility shift assays showed the Myb protein could not recognize the modified sequence as its binding structure.

The possibility of introducing 5-formyl pyrimidine nucleosides into the Myb binding sequence is currently under investigation to target an ε-amino group of a certain lysine residue in Myb.

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2. It was found that 2'-deoxy-6-(1,2-diacetoxy)ethyluridine was unstable during storage at ambient temperature. For a leading reference on 2'-deoxy-5-(1,2-diacetoxy)ethyluridine, see: Sugiyama, H.; Matsuda, S.; Kino, K.; Zhang, Q. -M.; Yonei, S.; Saito, I. *Tetrahedron Lett.* **1996**, *37*, 9067-9070.
3. Ross, B. S.; Springer, R. H.; Tortorici, Z.; Dimock, S. *Nucleosides Nucleotides* **1997**, *16*, 1641-1643. Chemical yield is described in parentheses for each step.
4. The modified Myb binding sequence is underlined.
5. The oligo was treated with phosphodiesterase and alkaline phosphatase in 50 mM phosphate buffer (pH 7.2) at 37 °C for 5 h. HPLC analysis on a PRODIGY ODS column (150×4.6 mm, 40 °C, eluted with 50 mM HCO_2NH_4 buffer at a flow rate of 1.0 mL/min and detected at 260 nm) showed the peak of 6-formyl-2'-*O*-methylcytidine with the retention time of 7.4 min.