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Synthesis and binding property of an oligonucleotide containing tetrafluorophenoxazine

Jianying Wang, Kuei-Ying Lin, and Mark D. Matteucci*

Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, California, 94404, USA

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Abstract: A tricyclic pyrimidine nucleoside analog, termed tetrafluorophenoxazine, has been synthesized and incorporated into an oligonucleotide. Tm analyses demonstrate that this analog is capable of enhanced recognition of both a complementary adenine and guanine within a DNA helix.

Keywords: Nucleic acid analogs; tautomerism

The Watson-Crick base pairing interactions within helical nucleic acids duplexes possess exquisite specificity.[1] This specificity is desirable in most applications of oligonucleotides (ONs) such as hybridization probes, PCR primers and gene inhibition through an antisense mechanism. There are however specific applications in which base analogs which hybridize without specificity are useful. The first synthetic analogs of this type were the N-alkoxycytosine nucleosides which could pair as a thymine or cytosine because of a tautomeric equilibrium.[2] Subsequently, bases capable of stacking but devoid of hydrogen bonding function have become useful in hybridizations and PCR reactions where only a partial complementary DNA sequence is known.[3,4] All such analogs to date have shown reduced affinity when paired with a "complementary" adenine (A), guanine (G), cytosine (C) or thymine (T) within a helix. This lower affinity limits the number of such analogs which can be incorporated into a hybridization probe or PCR primer.[4]

We have identified a tricyclic pyrimidine analog, termed tetrafluorophenoxazine, which binds with nearly equal affinity to both a complementary A and G. These pairings are of enhanced affinity relative to both an AT and GC base pair. This derivative is based on our previous work with the parent tricyclic pyrimidine nucleoside, phenoxazine.[5]

Synthesis of the tetrafluorophenoxazine nucleoside **5** can be effected in a simple manner using a double nucleophilic aromatic substitution reaction in the key step as shown in Scheme 1. Bromination of 2'-deoxycytidine hydrochloride **1** followed by *in situ* base treatment with N,N-diisopropylethylamine provided 5-hydroxyl-2'-deoxycytidine **2**.[6] The tetrafluorophenoxazine nucleoside **3** was obtained by cyclization of **2** with hexafluorobenzene in DMSO in the presence of potassium

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This ability to recognize both A and G with enhanced affinity could find applications in the field of the regulation of gene expression by the antisense approach. Such an example would be the targeting of the initiation of translation region of the gag gene in HIV 1.[10] The sequence of this region is highly conserved between HIV strains with only a variation at nucleotide 18.[11] The nucleotide at this position can either be an A or a G in the target mRNA.[12] Tetrafluorophenoxazine containing antisense ONs maybe able to target both sequences with high affinity.

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