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Synthesis and binding property of an oligonucleotide containing tetrafluorophenoxazine

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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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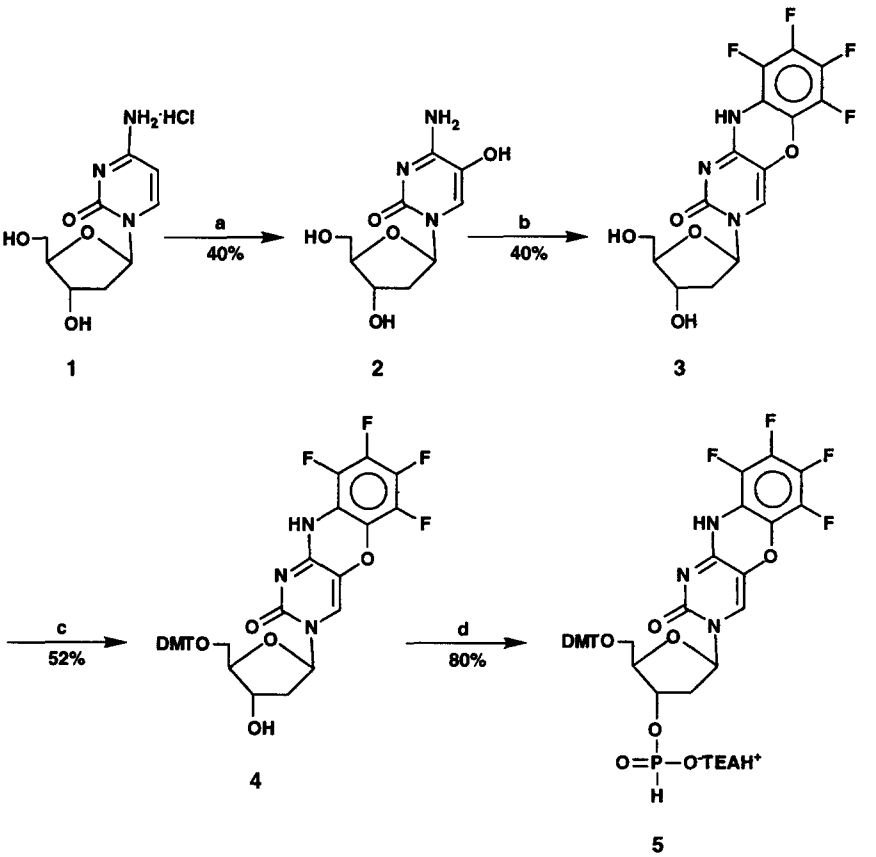
carbonate. Dimethoxytritylation and subsequent phosphitylation[7] of **3** generated the H-phosphonate **5**. Compound **5** was incorporated into the 15-mer ON, **9**, shown in Table1 via solid-phase DNA synthesis using a H-phosphonate protocol[8] and characterized by MALDI-TOF mass spectrometry.[9]

Scheme 1. Synthesis of Tetrafluorophenoxazine Nucleoside 5



a: (1) bromine, H_2O, room temp.(rt); (2) N,N-diisopropylethylamine; b: K_2CO_3, hexafluorobenzene, DMSO, 50 °C; c: DMTCl, pyridine, rt.; d: 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one, pyridine, CH_2Cl_2, 0 °C to rt.

The binding properties of ON **9** and appropriate controls to complementary DNA were determined by Tm analysis. The data are shown in Table 1.

Table 1. Tm Study of ONs

DNA Target **6**: 3'-AGAG**X**GAGAGAAAAA

ON	ON Sequences	Tm °C TARGET X=G	Tm °C TARGET X=A	Tm °C TARGET X=T	Tm °C TARGET X=C
C^m control, **7**	5'-$TC^mTC^m\mathbf{C^m}C^mTC^mTC^mTTTTT$	57.5	45.0	44.5	43.0
		highest	(-12.5)	(-13.0)	(-14.5)
T control, **8**	5'-$TC^mTC^m\mathbf{T}C^mTC^mTC^mTTTTT$	49	53.5	46.0	44.0
		(-4.5)	highest	(-7.5)	(-9.5)
P^F, **9**	5'-$TC^mTC^m\mathbf{P^F}C^mTC^mTC^mTTTTT$	59.0	58.0	49.0	50.0
		highest	(-1.0)	(-10.0)	(-9.0)

Values in parentheses are the ΔTm between the observed Tm and highest Tm for that ON.

T is thymidine. C^m is 5-methyl-2'-deoxycytidine. P^F is tetrafluorophenoxazine. All linkages are phosphodiester. Tm data were measured in a buffer solution of 140 mM KCl / 5 mM Na_2HPO_4 / 1 mM $MgCl_2$ at pH = 7.2, and the concentration of all ONs was about 2 μM. Error in Tm measurements was 0.5 °C.

Both the C^m and T containing control ONs are specific for G and A, respectively. Mismatches are discriminated in the case of **7** (C^m) by 12.5 to 14.5 °C with the least discrimination occurring when being paired with A. Mismatch discrimination is less pronounced in the case of the T containing ON. ON **8** showed mismatch discriminations of 4.5 to 9.5 °C with the least discrimination being with G. The tetrafluorophenoxazine ON **9** discriminated well when paired with the pyrimidines, T and C, (10 and 9 °C, respectively) but showed almost identical recognition of the purines, G and A. This purine recognition is significant in that the affinity is superior to both the AT and GC perfect matches.

The reason for the lack of discrimination between G and A with tetrafluorophenoxazine is likely due to a ΔG of near zero between the amino (C like) and imino (T like) tautomeric forms of the base as was previously observed with N-methoxycytosine.[2] This shift to near zero, must be effected by the four fluorine atoms on the third ring in the tricycle. The parent phenoxazine exists largely in the C like state as evidenced by its selectively for pairing with G.[5] The enhanced affinity for both A and G is likely due to enhanced stacking interactions with adjacent bases as has previously been observed with the parent phenoxazine.[5] This ability to shift the tautomeric equilibrium using substitution on the third ring raises the possibility of creating analogs of phenoxazine which are T analogs with specific and enhanced affinity for A.

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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References and Notes:

1. Watson, J. D. and Crick, F. H. C. *Nature* **1953**, *171*, 737.
2. Lin, P. K. T. and Brown, D. M. *Nucl. Acids Res.* **1989**, *17*, 10373.
3. (a) Nichols, R.; Andrews, P. C.; Zhang, P. and Bergstrom, D. E. *Nature* **1994**, *369*, 492. (b) Bergstrom, D. E.; Zhang, P.; Toma, P. H.; Andrews, P. C. and Nichols, R. *J. Am. Chem. Soc.* **1995**, *117*, 1201.
4. Loakes, D. and Brown, D. M. *Nucl. Acids Res.* **1994**, *22*, 4039.
5. Lin, K.-Y.; Jones, R. J. and Matteucci, M. D. *J. Am. Chem. Soc.* **1995**, *117*, 3873.
6. Eaton, M. A. W. and Hutchinson, D. W. *Biochim. Biophys. Acta.* **1973**, *319*, 281.
7. Marugg, J. E.; Tromp, M.; Kuyl-Yehskiely, E.; van der Mared, G. A.; and van Boom, J. H. *Tetrahedron Lett.* **1986**, *27*, 2661.
8. (a) Froehler, B. C.; Ng, P. G. and Matteucci, M. D. *Nucleic Acids Res.* **1986**, *14*, 5399. (b) Froehler, B. C. in *Protocols for Oligonucleotides and Analogs: Synthesis and Properties*; Agrawal, S., Ed.; Humana: Totowa, NJ **1993**, pp 63-80.
9. MALDI MS: 4643.9 (calcd MH^+ = 4643.1)
10. Lisziewicz, J.; Sun, D.; Weichold, F.; Thierry, A.; Lusso, P.; Tang, J.; Gallo, R. and Agrawal, S. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 7942.
11. Nucleotide numbering starts from the AUG translation initiation codon of GAG.
12. *Human Retrovirusis and AIDS*, Myers, G.; Korber, B.; Berzofsky, J.; Smith, T.F. and Pavlakis, G., Eds; Los Alamos: Los Alamos National Laboratory **1992.**