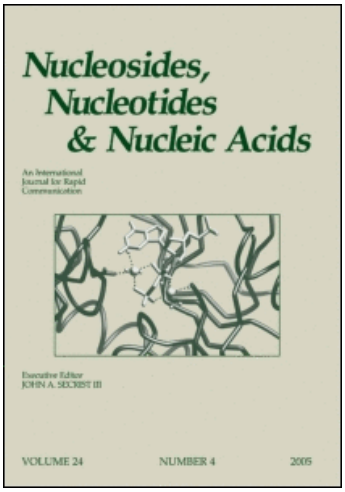

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Base Analogue Interactions in DNA Duplexes

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BASE ANALOGUE INTERACTIONS IN DNA DUPLEXES

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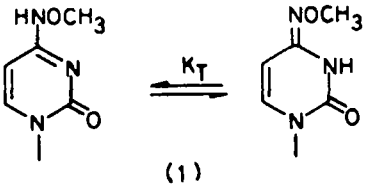
Abstract. Oligodeoxyribonucleotides containing N^4-methoxycytosine (mo^4C) and its 5-methyl derivative (mo^4m^5C) are synthesised and used to compare the stabilities of duplexes containing $mo^4C.A$ and $mo^4C.G$ base-pairs with those containing normal and mismatch pairs.

The design of oligonucleotide probes based on protein aminoacid sequences is complicated by the redundancy of the genetic code. Mixed probes in which all codon assignments are taken into account are normally used[1]. Multiple-chain probes are effective, but they may present problems that are difficult to assess in respect of purification, whether all species are present and, finally, diminished sensitivity. There have been attempts to circumvent these problems by insertion of "no base" (H) or "no-hydrogen bond" (aryl) or "wobble base" (hypoxanthine) analogues at degenerate sites[2,3,4]. The latter has been used in a number of cases but its advantage is not clear[5].

A more fundamental approach to the problem would utilize base analogues which were degenerate in their hydrogen-bonding potential. These might best be sought in ring-systems that had the requisite tautameric forms of approximately equal stability. The tautomeric equilibria and hydrogen bonding potential of N^4-hydroxy-, N^4-methoxy-cytosines (1) have been particularly well studied having K_Ts around 10-30 in favour of the imino-form[6,7]. N^4-Methoxy-derivatives were chosen for initial study.



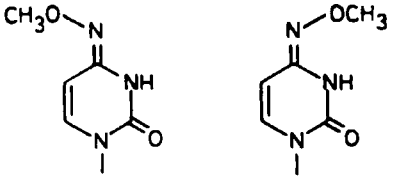
(1)

N^4-Methoxydeoxycytidine is readily synthesised from deoxyuridine via the 3', 5'-di-O-acetyl-4-methylthio- or a 4-triazolo-derivative. The 5-methyl analogue is available, likewise, from thymidine. These were converted to their 5'-dimethoxytrityl derivatives and thence to the corresponding 3'-O-(2-chlorophenylphosphates). The methoxyamino group was shown to be stable to the conditions used in the synthesis of oligonucleotides by the phosphotriest method: the dinucleoside phosphate mo^4m^5CT was synthesised and fully characterised by high field n.m.r. and FAB m.s.

For the stability of duplexes containing mo^4C and mo^4m^5C the following oligonucleotides, inter alia, were synthesised (Table 1). The T_mS and other thermodynamic parameters were derived from recorded melting transitions of these duplexes by standard procedures[9], and are recorded in Table 2.

First we should notice that the mismatches G.T. and A.C. have considerably lowered T_ms, as expected. Secondly the A.mo^4C T_m exceeds that of the normal A.T containing duplex and moreover duplexes containing A.mo^4C and G.mo^4C are of similar stabilities. In fact the later two 17-mers have remarkably sharp melting transitions, which is also associated with an increase in ΔG values over those for the A.T. and G.C. duplexes, indicative of an increase in cooperativity.

It is also noteworthy that both the A.mo^4m^5C and G.mo^4m^5C duplexes have very low T_m values. Evidence in the literature suggests a favoured syn conformation (2) for the methoxy group in N^4-methoxycytosine



(2)

derivatives[6,10]. We assume on the basis of our data that in the duplexes with mo^4C the methoxy group takes up the anti conformation on base-pairing. This, evidently, is sterically inhibited by the 5-methyl substituent in the mo^4m^5C containing duplexes with consequent reduction in stability.

We had available two M13 clones carrying the sequences corresponding to oligomers A and G (the one being a single site mutant

Table 1

Oligomer	Sequence (5'-3')
A	ACTTGGCC A CCATTTTG
G	ACTTGGCC G CCATTTTG
T	CAAAATGG T GGCCAAGT
C	CAAAATGG C GGCCAAGT
mo^4C	CAAAATGGmo^4C GGCCAAGT
mo^4m^5C	CAAAATGGmo^4m^5CGGCCAAGT

Table 2

Type of Duplex	T_m °C	T_d (range in °C)
A.T.	57	53-56
G.T.	51	45-47
G.C.	62	<55
A.C.	48	45-47
A.mo^4C	61	55-57
G.mo^4C	59	52-55
A.mo^4m^5C	39	<40
G.mo^4m^5C	38	<40

of the other), kindly provided by Dr. P. Carter. Dot-blot experiments using ssDNA from these bacteriophage, set out alternately on a nitrocellulose sheet in the form of a grid were carried out. Washing at elevated temperatures in the usual way[11] gave a measure of the dissociation temperature, T_d and the values are given in Table 2. The same order is observed as was found for the T_m values.

At least, then, in these experiments it is clear that N^4-methoxycytosine can act as a degenerate base residue, effectively standing in place of both cytosine and thymine.

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