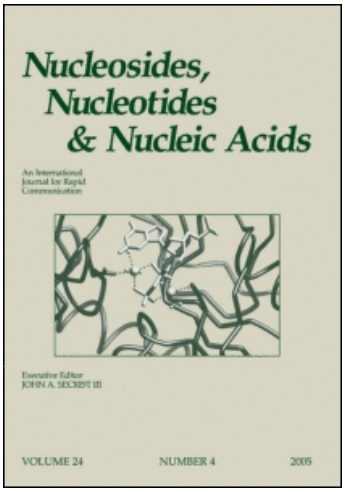

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Synthesis and Stability of Oligonucleotides Containing Purine Base Analogues

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SYNTHESIS AND STABILITY OF OLIGONUCLEOTIDES CONTAINING PURINE BASE ANALOGUES.

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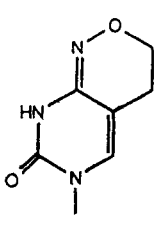
Abstract - The synthesis of the purine nucleoside analogues N^6-methoxydeoxyadenosine **3a** and the 9-deoxyribosyl derivative of the N^6-methoxy-2,6-diaminopurine **3b**, their introduction into oligomers and the stabilities of duplexes in which these are base-paired with T and C are described.

In a recent publication[1] we reported on the synthesis of a number of oligodeoxynucleotides containing the pyrimidine base analogue N^4-methoxycytosine (**M**) and a related oxazino-bicyclic derivative **1**, (**P**). Duplexes (17-mers) in which these bases were paired with A and G showed that the base pairs **P:A** and **P:G** showed comparable T_m values even with three such pairs. **M:A** and **M:G** pairs showed somewhat lower stability.

In extending these studies to include purine analogues, we have incorporated the residues N^6-methoxyadenine (**Z**) and N^6-methoxy-2,6-diaminopurine (**K**) into oligomers and studied their pairing with T and C. The rationale was that mo^6A (**Z**) shows degeneracy, i.e has a K_T value nearer to unity than A[2,3] and might form base pairs of type **2** and **3**. The tautomeric state of mo^6-diaminopurine, mo^6DAP, (**K**) has not been investigated.



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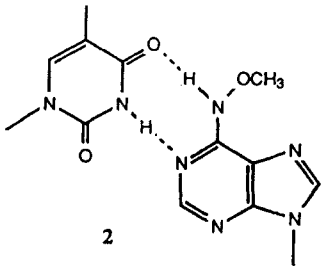

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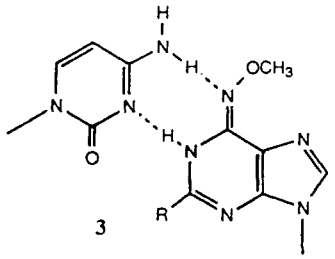

3

3a, R=H
3b, R=NH_2

The syntheses of the deoxynucleosides (**3a,b**) were carried out by coupling the corresponding 6-chloropurines with α-3,5-di-p-toluoyl-2-deoxyribosylchloride under the phase transfer conditions of Seela and co-workers.[4] Nucleosides (**3a,b**) have also been

TABLE. Melting transitions of heptadecamer duplexes

N^6-methoxyadenine (**Z**)	(°C)	N^6-methoxy-2,6-diaminopurine (**K**)	(°C)
ACTTGGCCACCATTTTG TGAACCGGTGGTAAAAC	70	ACTTGGCCGCCATTTTG TGAACCGGCGGTAAAAC	75
ACTTGGCCZCCATTTTG ----------T----------	65	ACTTGGCCKCCATTTTG ----------T----------	67
ACTTGGCCZCCATTTTG ----------C----------	64	ACTTGGCCKCCATTTTG ----------C----------	66
ACTTGGCCZCCZTTTTG ----------T---T---------	61	ACTTGGCCKCCKTTTTG ----------T---T---------	64
ACTTGGCCZCCZTTTTG ----------C---T---------	57	ACTTGGCCKCCKTTTTG ----------C---T---------	62
ACTTGZCCZCCATTTTG ----------C----C----------	52	ACTTGKCCKCCATTTTG ----------C-----C----------	59
ACTTGGCCACCATTTTG ---------T------C----C---------	43	ACTTGKCCKCCKTTTTG ----------C-----T-----T---------	58
		ACTTGKCCKCCKTTTTG ---------- C----C-----T----------	57

The transitions were measured at 260 nm in 6xSSC buffer at pH 6.7.

synthesised by Ueda and coworkers.[5,6] The conversion of those nucleosides (**3a**, **3b**) to the corresponding 5'-dimethoxytrityl-3'-amidites and their incorporation into oligomers using an automatic ABI DNA synthesiser and a Pharmacia Gene Assembler, then deprotected and purified by ion exchange hplc were done in the usual way.

The Table lists the T_m values of a series of duplexes containing one or more of the residues **Z** and **K**, and for comparison the corresponding true complements and a triple mismatch.

When a single A or G is substituted by either of the analogues mo^6A (**Z**) and mo^6DAP (**K**) there is a depression of 5-8° in T_m. However the stabilities of these duplexes containing **Z:T** and **Z:C** are essentially the same, as are those with **K:T** and **K:C**. The stabilities, it has been noted,[5] of two pentadecamers containing **Z:T** and **Z:C** pairs are of comparable stability. Additional base analogue substitutions lead to some decrease in T_m (more marked in the case of **Z**) but much less than a related mismatch (Table).

The nature of the base pair structures in these duplexes is not clear. N^6-methoxyadenine derivatives have been much studied and appear to have imino:amino ratios of around 80:20 in polar solvents from our own and others' measurements.[2,3] The small free energy difference should allow normal base pairs (**2** and **3**) with T and C. However the evidence that the methoxyl function shows a strong preference for *syn* geometry (with respect to N1)[7] may preclude this view, since **2** and **3** both require the *anti* configuration. The same configurational problem pertains in base pairs formed with mo^6DAP(**K**). In addition we find no nmr evidence for two tautomers in preliminary nmr experiments with **3b**.

We are seeking further information on the base pair structures involving **Z** and **K** but the duplex stabilities shown in the Table indicate that both purines derivatives form base pairs with C and T containing at least two hydrogen bonds. We note for comparison that mo^4C:G in a self complementary octamer duplex is a wobble pair with the methoxyl group in the *syn* configuration.[8]

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