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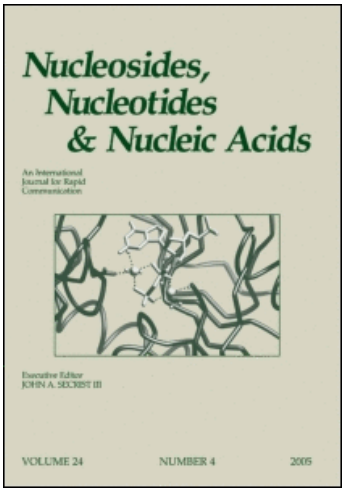

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Pyrazolo[3,4-*d*]pyrimidine Nucleic Acids: Adjustment of the dA-dT to the dG-dC Base Pair Stability

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ABSTRACT

The pyrazolo[3,4-*d*]pyrimidine-4,6-diamine nucleosides **2b-d** stabilize the dA-dT base pair significantly when the dA-residue is replaced. Oligonucleotide duplexes incorporating **2b-d** show a 4–6°C T_m increase per modification. The 7-bromo compound **2b** harmonizes the stability of the dA-dT vs. the dG-dC pair. According to this the stability of such duplexes depends no longer on the base pair composition of a DNA molecule.

Key Words: Nucleosides; Pyrazolo[3,4-d]pyrimidine; DNA; Base pairing; Purine-2,6-diamine.

INTRODUCTION

The guanine-cytosine pair is more stable than the adenine-thymine pair which is mainly attributed to the formation of a third hydrogen bond. Oligonucleotide duplexes forming other tridentate base pairs are expected to show a similar behavior. However, the additional amino group of 2-amino-2′-deoxyadenosine (**1**)[1,2]

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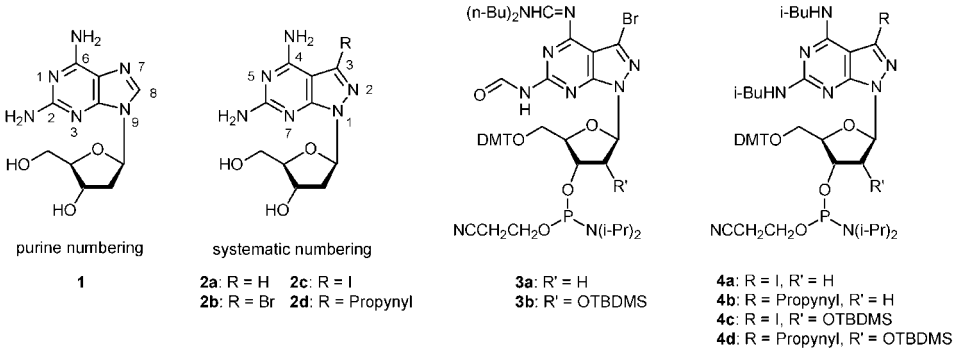

Scheme 1.

stabilizes the dA-dT pair only very little resulting in a T_m increase of a 12-mer oligonucleotide duplex by only 1−2°C per modification.[3] This unusual behavior[4] prompted us to search for more stable tridentate base pair with a dA-dT base recognition pattern. The problem was approached by replacing the purine moiety of compound **1** by a 8-aza-7-deazapurine (pyrazolo[3,4-*d*]pyrimidine) system. This heterocycle mimics the shape of the purine and can substitute canonical nucleobases efficiently (**2a** vs. **1**). As it was found that 7-halogeno- or 7-alkynyl substituents (purine numbering is used throughout) increase the base pair stability[5] the favorable properties of the modified base were combined with those of the 7-substituents. This work reports on oligonucleotides incorporating the nucleosides **2b-d**. Oligonucleotides are accessible via the phosphoramidites **3a,b** or **4a-d** (Sch. 1). It will be shown that compound **2b** harmonizes the base pair stability of dA-dT and dG-dC.[6]

RESULTS AND DISCUSSION

1. *Monomers.* The isopropoxy nucleosides **5b-d** served as precursor molecules for the synthesis of the 8-aza-7-deazapurin-2,6-diamine (pyrazolo[3,4-*d*]pyrimidin-4,6-diamine) nucleosides **2b-d**.[3,7] The former were prepared by the stereoselective nucleobase anion glycosylation developed in our laboratory.[8] The propynyl nucleoside **2d** was obtained by the Pd-assisted Sonogashira cross coupling reaction from the iodo nucleoside **2c**.[7] The *N,N*-di-n-butylaminomethylidene group was used for the protection of the bromo nucleoside **2b**. The bis-amidine **6** was formed together with the formyl derivative **7a**. The reaction was completed by hydrolysis of the reaction mixture to give **7a,** exclusively. From the half-life values of deprotection (aq. NH_3) it became evident that the combination of these protecting groups was optimal for further manipulations. Subsequently, the 5′-hydroxyl group was protected with the 4,4′-dimethoxytrityl residue to give **7b** (Sch. 2). Phosphitylation furnished the phosphoramidite **3a**. This compound as well as the corresponding phosphoramidites **4a,b** were employed in solid-phase oligonucleotide synthesis.

2. *Oligonucleotides.* The nucleosides **2a-d** were incorporated into the non-self-complementary duplex 5′-d(TAGGTCAATACT) • 5′-d(AGTATTGACCTA) by solid-phase synthesis using phosphoramidite chemistry. In all cases an extraordinarily

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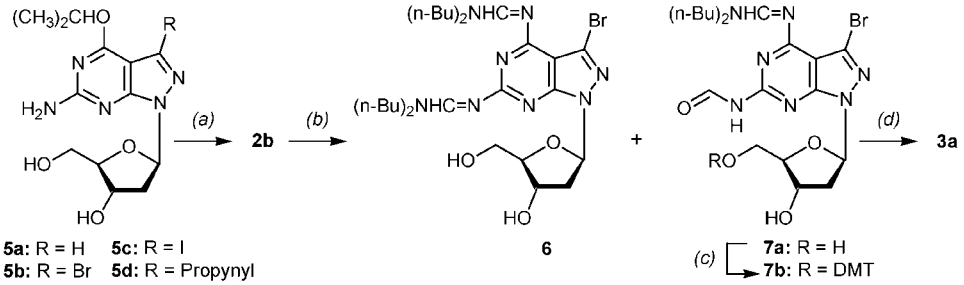

Scheme 2. (a) **5b** → **2b** aq.NH_3, autoclave; (b) *N,N*-di-n-butylformamide dimethyl acetal, MeOH; (c) **7a** → **7b** (DMTCl, pyridine); (d) **7b** → **3a** $(NCCH_2CH_2O)(iPr)_2NPCl$, $(iPr)_2EtN$, CH_2Cl_2.

strong duplex stabilization was observed.[3,6,7] A 4–6°C T_m increase was found when the **2b**-dT pair was substituting a dA-dT pair. By comparing the T_m values of duplexes incorporating **2b**-dT base pairs in place of dG-dC (Table 1) it becomes apparent that the novel base pair develops the same stability as the dG-dC pair. This is the case for single or multiple incorporations or for replacements at various positions (Table 1). This phenomenon can be explained by

(i) The formation of the third hydrogen bond.
(ii) The higher polarizability of the modified vs. the unmodified base.

Table 1. Comparison of T_m values and thermodynamic data of oligonucleotides incorporating **2b**-dT or dG-dC base pairs.

Duplex	T_m [°C]	ΔH° [kcal/mol]	ΔS° [cal/mol K]	ΔG°_{310} [kcal/mol]
5′-d(TAG GTC **2b**AT ACT) 3′-d(ATC CAG **T**TA TGA)	54	−100	−281	−12.9
5′-d(TAG GTC **G**AT ACT) 3′-d(ATC CAG **C**TA TGA)	53	−89	−248	−12.1
5′-d(TAG GTC **2b2b**T ACT) 3′-d(ATC CAG **TT**A TGA)	56	−91	−252	−13.4
5′-d(TAG GTC **GG**T ACT) 3′-d(ATC CAG **CC**A TGA)	57	−104	−290	−14.1
5′-d(TAG G**T**C AA**T** ACT) 3′-d(ATC C**2b**G TT**2b** TGA)	59	−92	−251	−14.0
5′-d(TAG G**C**C AA**C** ACT) 3′-d(ATC C**G**G TT**G** TGA)	58	−98	−270	−14.1
5′-d(T**2b**G GTC **2b2b**T **2b**CT) 3′-d(A**T**C CAG **TT**A **T**GA)	64	−112	−305	−17.1
5′-d(T**GG** GTC **GG**T **G**CT) 3′-d(A**CC** CAG **CC**A **C**GA)	64	−110	−302	−16.9

Measured at 260 nm in 0.1 M NaCl, 10 mM $MgCl_2$, and 10 mM Na-cacodylate buffer, pH 7.0 with 5 μM + 5 μM single strand concentration.

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(iii) The hydrophobic character of the 7-substituent expelling water molecules from the major groove and
(iv) The –I effect of the 7-substituents increasing the proton donor properties of the amino groups.

CONCLUSION

The replacement of dA-residues by the halogenated nucleoside **2b** adjusts the stability of a dA-dT base pair to that of a dG-dC pair. This modification maintains the sequence specificity of an oligonucleotide during hybridization and shows similar base discrimination as dA. Application of this phenomenon to sequence specific hybridization protocols performed in solution or on oligonucleotides immobilized on polymeric surfaces (DNA arrays) is under investigation.

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