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Synthesis and incorporation into DNA fragments of the artificial nucleobase, 2-amino-8-oxopurine

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Abstract—The nucleoside 2-amino-9-(2-deoxy-β-D-ribofuranosyl)-7,8-dihydro-8-oxo-purine (dJ) was obtained in eight steps from 2′-deoxyguanosine. The appropriate protected phosphoramidite was synthesized and incorporated into DNA oligonucleotides. The thermal stability of heteroduplexes containing 2-amino-8-oxopurine (J) was investigated by UV-thermal denaturation experiments. The results obtained can be interpreted by the base pairing schemes involving the two edges of dJ depending on the *anti* and *syn* orientations.

Faithful transmission of hereditary messages by nucleic acids depends upon the pairing between a large bicyclic purine and a smaller monocyclic pyrimidine both in the *anti* conformation according to a scheme known as the Watson–Crick geometry.[1] However there is no reason to assume that the requirements for duplex stability and replication must limit the genetic alphabet to only two base pairs. Efforts to expand the genetic alphabet to a third base pair have resulted during the last decades in the development of several candidates. Works with modified nucleobases have explored not only hydrogen-bonded pairs[2–5] but also nonpolar pairs[6,7] and metal-mediated pairs.[8] In addition to heteropairs, several promising self-pairs have been identified, among them, purine–purine pairs have been designed.[9,10] More recently, new base pairing motifs in which building blocks are size-expanded into tricyclic bases have been reported.[11,12]

Purine nucleosides are inherently ambivalent, capable of flipping into the *syn* glycosidic conformation in addition to the *anti* conformation and thus of displaying either of two edges for hydrogen bonding with other bases.[13] Certain purine pairs are indeed genetically encoded, such as the conserved A(*syn*):G(*anti*) pairs present in helices of ribosomal and transfer RNA secondary structures.[14] The formation of other purine pairs is actively avoided by organisms, the most documented case being the repair process for correcting 8-oxodG(*syn*):dA(*anti*) pairs formed during erroneous replication involving 8-oxodGMP.[15] The mutagenic properties of 8-oxodG[16] reflect the stability of the lesion when paired in the *syn* conformation with dA(*anti*). Starting from the 8-oxoguanine(*syn*):adenine(*anti*) mispairing scheme (Fig. 1a), we designed an artificial nucleobase, 2-amino-8-oxopurine (noted J), potentially able to form base pairs in the *syn* or *anti* conformation.

As the 8-keto form predominates at physiological pH, dJ possess a hydrogen bond donor at the 7 position and a hydrogen bond acceptor at the 8 position, coupled with the hydrogen bond pattern of 2-amino-purine on the Watson–Crick side. Thus, J in the *anti* conformation presents its 2-aminopurine edge for pairing and can form two hydrogen bonds with base T according to Watson–Crick geometry (Fig. 1b). Other pairings with C and A involve the formation of less favored protonated Watson–Crick or neutral Wobble pyr–pur and large Wobble pur–pur base pairs. Such hydrogen bonds between 2-aminopurine and A or C have been supported by NMR,[17,18] however only AP:C base pair is involved in its mutagenic profile.

Keywords: Nucleobase; Nucleoside; Conformation; Base pairing; DNA.
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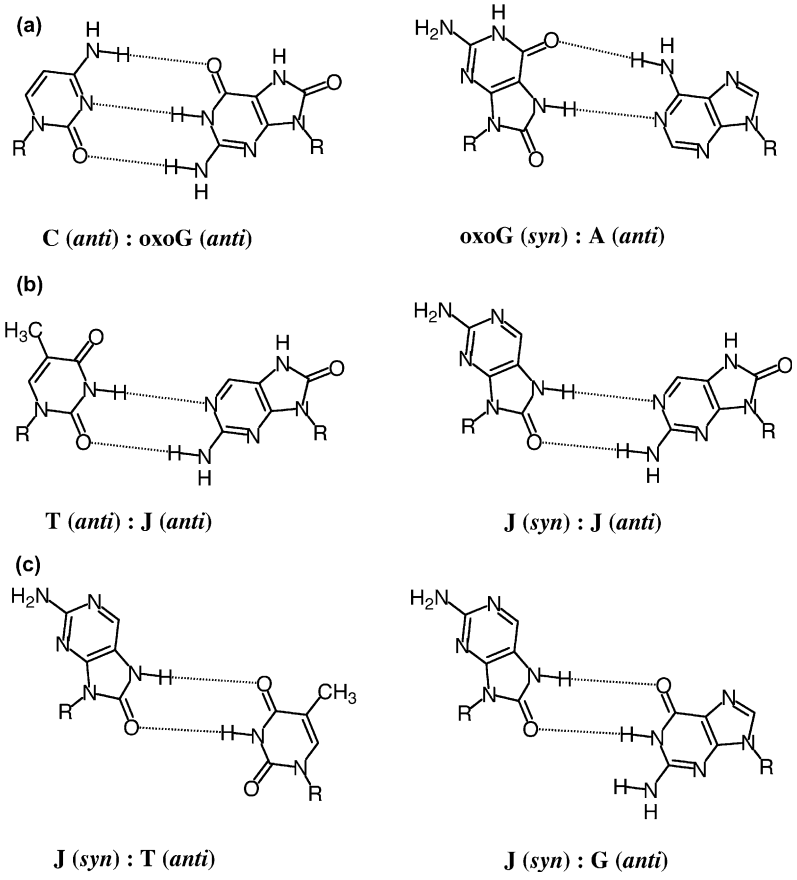

Figure 1. Proposed structures of hydrogen-bonded base pairs involving 2-amino-8-oxopurine (J) compared to those of 8-oxoguanine (oxoG).

The absence of any donor or acceptor character at the 6 position changes the pairing of J in the *syn* conformation compared to oxoG(*syn*). However, as the result of the 7-NH donor and 8-oxo acceptor characters, J(*syn*) can pair with itself in the *anti* configuration with a shape similar to the oxoG(*syn*):A(*anti*) pair (Fig. 1b). Hydrogen bonded pairs with T and G result in a displacement of the purine J in the *syn* conformation compared to J(*anti*):J(*syn*) pair (Fig. 1c).

Nucleoside **1** was synthesized in eight steps from 2′-deoxyguanosine (dG) according to a previous report with slight modifications.[19] We have selected the benzoyl group for the protection of hydroxyls and exocyclic amine to improve purification and global yield. The main steps are outlined in Scheme 1 and nucleoside **1** was isolated in 6% global yield. Nucleoside **1** was fully characterized by elemental analysis, NMR and mass spectrometries.[20] The UV absorbance spectrum of **1** in water (pH 7.2) exhibited a maximum at 240 nm (ε 8970) and at 309 nm (ε 7100), characteristic of 2-aminopurine derivatives (fluorescence).

The conformational analysis of the furanose puckering of **1** was determined by means of the sums of proton–proton coupling constants.[21] The fraction *S*-type conformer (p*S*) of **1** determined by ^{1}H NMR at 400 MHz was 65%. This preference indicates a rapid dynamic equilibrium between *N* and *S* states in solution.

The orientation of the aromatic base with respect to the sugar moiety is determined by the glycosidic torsion angle χ defined as the O-4′-C-1′-N-9-C-4. A number of methods have been developed to determine the χ values using NMR data. Although quantitative evaluation of interproton distances by mean of NOE measurement is widely used most often, in absence of proton at C8 position the values of χ are determined from the measurement of three-bond carbon–proton scalar couplings $^3J_{CH}$ across the glycosidic bond and DFT analysis using Karplus equations.[22] The $^3J_{C4-H1'}$ and $^3J_{C8-H1'}$ of nucleoside **1** were determined at 30 mM in D_2O (pH 7.2) by gradient selected ^3J-HMBC NMR experiments recorded at 600 MHz. The experimental values of three-bond carbon–proton scalar couplings $^3J_{CH}$ of **1** were 4.29 Hz for $^3J_{C4-H1'}$ and 3.77 Hz for $^3J_{C8-H1'}$. Comparison of these values with the $^3J_{C4-H1'}$ and $^3J_{C8-H1'}$ values reported for purines[22] and taking into account that both $^3J_{C4-H1'} > {}^3J_{C8-H1'}$ and ΔJ ($^3J_{C4-H1'} - {}^3J_{C8-H1'}$) is small[23] allowed us to conclude that the nucleoside **1** adopts in solution a preferential *syn* conformation. As described for adenine and guanine nucleobases, the introduction of a bulky group on C8 of 2-aminopurine moiety results in the increased of the *syn* population and should promote the pairing mode of 2-amino-8-oxopurine (J) in *syn* conformation.

For the preparation of the phosphoramidite derivative **5**, both the 2-amino and 7-imino functions were