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5-Amino-dU: A Nucleoside Analogue in the Central Strand of DNA Triplex for Orientation Selective Binding of A/G/C/T in the Third Strand†

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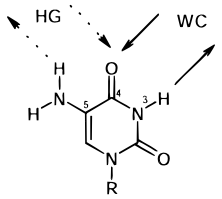
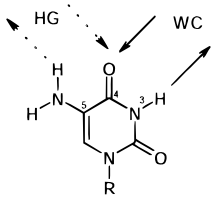
ABSTRACT



5-Amino-dU, a designed nucleoside analogue, when placed in the central strand of DNA triple helix, recognizes all four bases A, G, C, and T in the third strand, with a selectivity based on the orientation (parallel/antiparallel) of the third strand.

DNA triplexes originate from binding of a third strand of either pyrimidine (Y) or purine (R) rich oligonucleotide in the major groove of double helix, in parallel (*p*) or antiparallel (*ap*) orientation, respectively.[1] The specificity in triplex formation is derived from Hoogsteen hydrogen bonding by which T recognizes A of the A:T base pair (T*A:T) and protonated C^+ binds to G of the G:C base pair (C^+*G:C) in the pyrimidine motif. Similarly, in the purine motif, the third strand A binds to A of A:T, while G binds to G of the G:C base pair by reverse Hoogsteen mode. A common feature of all the above triads is the requirement of a purine (A or G) in the central position, as only these can form hydrogen bonding from both sides. This prompted a search for chemically modified nucleoside analogues that form stable triplexes,[2] and among these, φ and *iso*-φ nucleobases of C and U are capable of forming hydrogen bonds from both sides and hence are compatible in the central position of a triplex triad.[3] We recently demonstrated that 5-amino-dU ($U^{\#}$), an analogue of naturally occurring nucleoside dU is a



5-amino-dU ($U^{\#}$)

purine mimic and when present in the central strand can specifically recognize the purines in a third strand, A in parallel and G in antiparallel orientation (Figure 1).[4]

This Letter reports the selectivity in recognition of third

† NCL Communication No. 6468. Abbreviations: X*Y:Z indicates the three strands of triplex in which * and : represent Hoogsteen and Watson-Crick hydrogen bonding patterns, respectively. *p*, parallel; *ap*, antiparallel.

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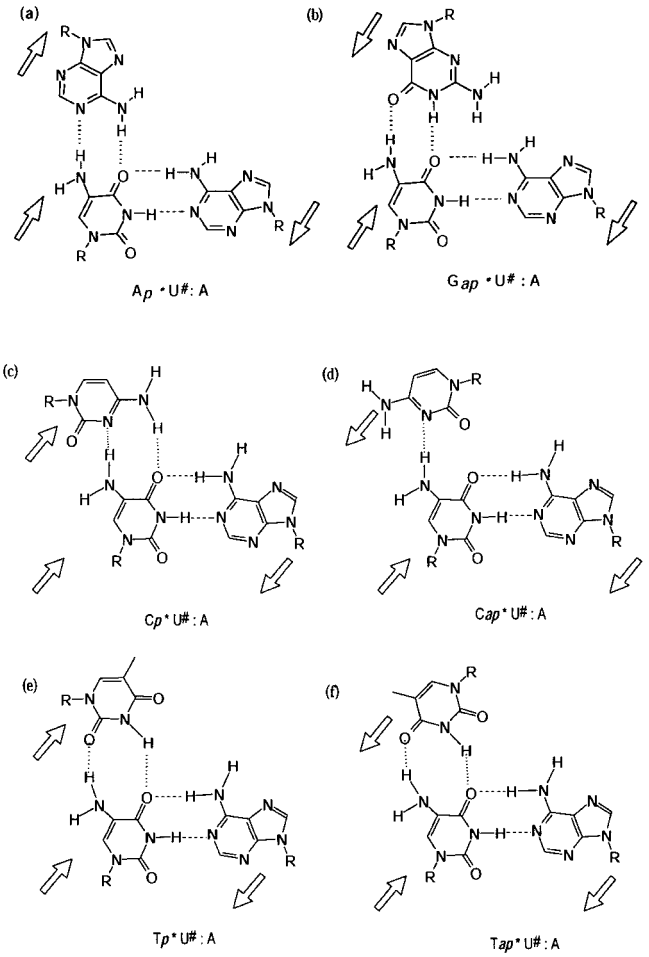

Figure 1. Hydrogen bonding schemes for 5-amino dU ($U^{\#}$) in the central strand.

strand pyrimidines C and T by $U^{\#}$, which forms a triple helix with T in both pyrimidine (parallel) and purine (antiparallel) motifs, but with C only in the pyrimidine motif. With this finding, 5-amino-dU becomes the first analogue that recognizes all bases A, G, C, and T of the third strand, in a orientation specific manner, and further triplexes containing $U^{\#}$ are more stable than corresponding controls with T in an identical position.

5′-TTCTTTT X_p TTCTTCT-3′
3′-AAGAAAA X_{ap} AAGAAGA-5′
5′-C GAAGAAAA Y AAGAAGACC-3′
3′-GCTTCTTTT Z TTCTTCTGG 5′

1.X_p=C; **2**.X_p=T, **3**.X_{ap}=C; **4**.X_{ap}=T, **5**.Y=$U^{\#}$; **6**.Y=T, **7**.Z=A

The oligonucleotides **1**−**7** were designed to combinatorially constitute various desired triplexes and were prepared by standard procedures[5] using trifluoroacetyl as the 5-NH_2 protector. All oligonucleotides were purified by reverese phase HPLC for UV melting experiments, and the identity of $U^{\#}$ oligonucleotide **5** was established by its mass apectrum using MALDI-TOF ($C_{188}H_{211}N_{87}O_{105}P_{18}$, M_{calc} 5923.9, M_{obs}, 5923). UV melting experiments indicated the formation of a duplex (**7**:**5**) with T_m slightly lower than that of the control duplex (**7**:**6**) with T in place of $U^{\#}$. The various triplexes containing $U^{\#}$ in the central strand and the corresponding control triplexes with T were individually constituted by heating equimolar amounts (1 μM) of appropriate strands at 80 °C for 3 min in 100 mM sodium cacodylate (pH 5.8) containing 20 mM $MgCl_2$ and 1 M NaCl, followed by slow cooling. Successful formation of triplexes were characterized by well-defined biphasic transitions in UV absorbance vs temperature profiles (Figures 2 and 3), and accurate T_ms (Table 1) were obtained from the corresponding maximum in first derivative curves.

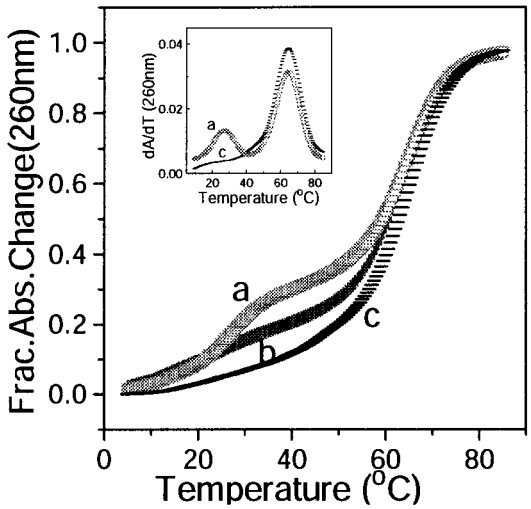

Figure 2. UV absorbance (260 nm) vs temperature profiles for (a) Cp*$U^{\#}$:A, pH 5.8; (b) Cp*$U^{\#}$:A, pH 7.1; and (c) Cap*$U^{\#}$:A, pH 5.8. Inset: first derivative curves for (a) and (c).

The parallel triplex having triad C_p*$U^{\#}$:A (**1***5:7) shows a higher UV T_m (ΔT_m = 6°) compared to the control triplex C_p*T:A (**1***6:7), (Table 1, entries 1 and 2) while no triplex was detected for antiparallel triplex C_{ap}*$U^{\#}$:A (Figure 2c). The parallel triplex formation was better at pH 5.8 (Figure 2a) than at pH 7.1 (Figure 2b), since the third strand C needs

Table 1. UV T_m of Triplexes[a]

entry	triad (X*Y:Z)	triplex	T_m (°C), pH 5.8
1	C_p*$U^{\#}$:A	**1***5:7	26
2	C_p*T:A	**1***6:7	20
3	T_p*$U^{\#}$:A	**2***5:7	26
4	T_p*$T^{\#}$:A	**2***6:7	19.5
5	T_{ap}*$U^{\#}$:A	**4***5:7	36

[a] No triplexes are seen with C_{ap}*$U^{\#}$:A (**3***5:7), C_{ap}*T:A (**3***6:7), and T_{ap}*T:A (**4***6:7); T_m values are accurate to ±0.5 °C.

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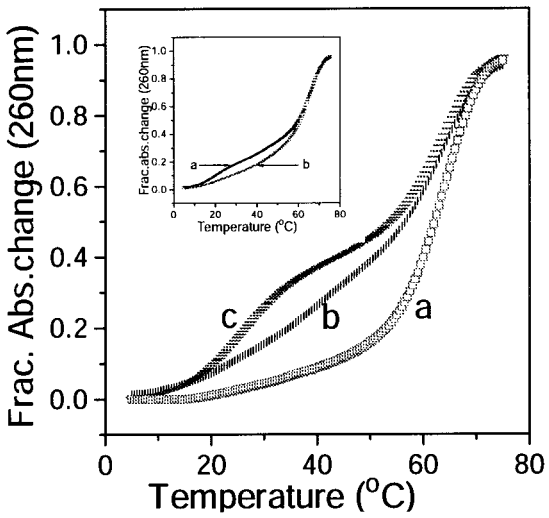

Figure 3. UV absorbance (260 nm) vs temperature profiles at pH 5.8 for (a) duplex U#:A and triplexes (b) T_{ap}*U#:A and (c) T_p*U#:A. Inset: UV melting curves for triplexes (a) T_p*T:A and (c) T_{ap}*T:A.

protonation for recognition of G. The observance of triplex with T in the middle strand (**1*6**:**7**) may be due to the one hydrogen bond possible between O4 of T with 4-NH_2 of C. When U# is in the central position, two hydrogen bonds are possible with C in the parallel orientation (Figure 1c), causing a higher T_m the for C_p*U#:A triplex, while only one hydrogen bond is possible in the antiparallel mode (Figure 1d).

In contrast to the selectivity observed for recognition of C by U# in the parallel mode, triplex formation is observed for T in the third strand in both pyrimidine (parallel, Figure 3c) and purine (antiparallel, Figure 3b) motifs. From the hydrogen bonding scheme, it is seen that the C2 carbonyl of T is involved in hydrogen bonding with 5-NH_2 of U# in parallel binding (Figure 1e) while the C4 carbonyl of T forms a hydrogen bond with U# in the antiparallel mode (Figure 1f). The parallel triplex with T in the middle strand (**2*6**:**7**) (Figure 3, inset, curve a) showed a lower T_m (ΔT_m = 6.5°) compared to that of U# (**2*5:7**) (Table 1, entries 3 and 4). No triplex formation was noticed for the corresponding antiparallel triplex containing T (Figure 3, inset, curve b) in the central position instead of U#, which exhibited triplex formation at pH 5.8 (Table 1, entry 5) and pH 7.1 (T_m = 38 °C). A slightly higher stability seen at pH 7.1 is in agreement with that expected for the purine motif.[1] The CD spectra of triplexes with U# (**1*5:7** and **2*5**:**7**) showed an intense negative band at 210−215 nm, characteristic of parallel triplexes,[6] and the general profiles of triplexes indicated no major structural changes induced by U# in the triplexes.

These experimental results along with our earlier work highlight the utility of 5-amino-dU (U#), a pyrimidine analogue designed as a purine mimic, in DNA triplex formation. When U# is present in the central strand, a remarkable orientation selectivity is observed in recognition of purines A/G and pyrimidines C/T. The triplexes formed with U# are also generally more stable than the control triplexes with T. In addition to providing the complementary hydrogen bond donor/acceptor sites, the observed stability could also be a consequence of alteration of the hydration pattern in the major groove by the hydrophilic 5-NH_2 group of U#. To our knowledge, this is the first example where a designed nucleoside analogue in the central strand recognizes all four bases A, G, C, and T with different selectivities based on parallel and antiparallel orientations. The use of modified φ bases in the central strand of the triplexes allows formation of triple stranded helices at single strand target sites of unrestricted sequence employing two oligonucleotide probes, one of which contains modified pyrimidines.[7] In this context, the present demonstration that a simple pyrimidine derivative 5-amino-dU in the second strand can selectively tolerate all four bases depending on orientation adds a new repertoire to nucleic acid recognition. Further work involves study of triplexes with multiple U# substitutions and explores other recognition tolerants of 5-amino-dU for modified bases in the third strand. U# is also emerging as a useful analogue for conjugation of reporter ligands.[8]

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Supporting Information Available: MALDI-TOF mass spectrum of **5** and UV melting profiles of T_{ap}*U#:A and T_{ap}*T:A and the corresponding first derivative profiles. This material is available free of charge via the Internet at http://pubs.acs.org.

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