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5-Propynyluracil·diaminopurine: an efficient base-pair for non-enzymatic transcription of DNA

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The Up·D base-pair (5-propynyl uracil·diaminopurine) is found to be more effective at non-enzymatic transcription than the corresponding natural T·A pair; under non-enzymatic reaction conditions where the natural T·A base-pair fails, a DNA template bearing Up efficiently directs the incorporation of D into a product RNA strand.

DNA and RNA are important models for prebiotic replication processes.[1] Of the two Watson–Crick base-pairs, G·C is known to outperform A·U(T) in abiotic copying assays.[2] This situation is due in part to the relatively weak acceptor·donor(·acceptor) [A·D(·A)] hydrogen bonding pattern found in the latter, whose diminished stability has been attributed to less favorable electrostatic interactions between the nucleobases.[3] In addition to base-pairing interactions, nucleic acids rely on cooperative base stacking to drive helix formation. Of the four natural nucleobases, U(T) is known to make the smallest contribution to helix stacking energy,[3a] a factor which also contributes to the under performance of the A·U(T) pair.

Our laboratory seeks to improve the abiotic replication characteristics of the A·U(T) base-pair by investigating properties of surrogate bases with increased hydrogen bonding or stacking potential. We have addressed pairing limitations by replacing U with pseudouridine (Ψ), a base that enables formation of triple helices incorporating A·Ψ·A, thereby increasing binding enthalpy.[4] Herein we report template-directed reactions with the A·U(T) surrogate D·U^{P}, a base-pair with improved stacking and pairing characteristics (Fig. 1).

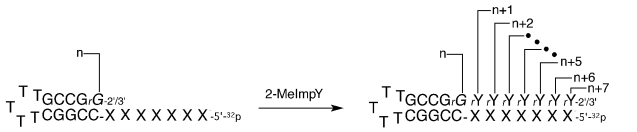
5-Alkynyl substituted pyrimidines have been known for some time to dramatically enhance the stability of nucleic acid helices.[5] Of the various alkynyl chains that have been investigated, propyne yields the most dramatic effect. Substitution of pyrimidine residues by 5-propynyl groups enhances *T*m's by 2.5 °C residue^{-1}.[6] We note that some pyrimidines with 5-substitutents have been posited to be as prebiotic as uracil itself.[7] We suggest that 5-propynylpyrimidines are models for modified pyrimidines that may have been present in early Earth environments.† ‡

The general template copying reaction used to assess the fitness of U^{P} for non-enzymatic information transfer is shown in Scheme 1. The template is a self-priming 5′-^{32}P-end-labelled hairpin of the type we have used in past studies to investigate copying reactions with non-standard nucleic acids.[8] Mononucleotides were activated with 2-methylimidazole, a group which confers sufficient reactivity to observe template reactions with DNA and RNA over the course of days in optimal cases.[2] As further indicated in Scheme 1, the template design is based on pyrimidine homo oligomers.

Results of template-directed synthesis on T or U^{P} containing homooligomer templates are shown in Fig. 2.§ Minimal oligomerization occurs on the natural dT$_7$ homooligomer template in the presence of either 2-MeImpA or 2-MeImpD as is evident from lanes 2 and 3 of this figure. In both lanes the predominant product after 10 days of incubation is unreacted template.

The non-standard dU$^{P}_7$ homooligomer gave results (Fig. 2, lanes 5 and 6) that contrast markedly with those noted above for the dT$_7$ template. Whereas the use of 2-MeImpA resulted in a poor oligomerization yield (lane 5), 2-MeImpD led to a high yield of highly extended oligomerization products (lane 6). As noted in the figure, the longer of the two main products in lane 6 derives from extension of the primer by six nucleotides, as deduced from time course data for the reaction (not shown).

The regiochemistry of the phosphodiester bond formed between the dU$^{P}_7$ template-primer and the first 5′-DMP residue incorporated, that between *n* and *n* + 1, was assessed using RNase T1[2,8] according to Scheme 2. This ribonuclease cleaves 3′,5′-phosphodiester linkages to the 3′-side of G residues. Lanes 4–6 of Fig. 3 show a single product after RNase T1 treatment of the oligomerization product from lane 6 of Fig. 2, a result that



Scheme 1 General non-enzymatic, template-directed oligomerization reaction used to evaluate base-pairs incorporating the nucleobases from Fig. 1. X = U^{P} or T, Y = D or A.

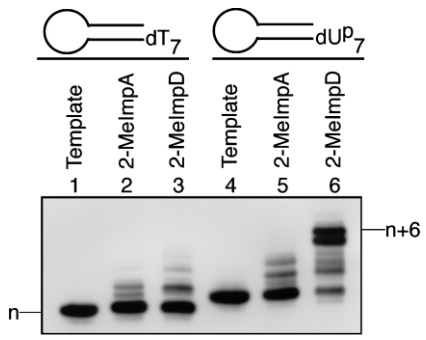


Fig. 2 Autoradiogram of 20% PAGE analysis of template-directed oligomerizations employing the indicated templates (see Scheme 1 for hairpin sequence) and mononucleotides. 2-MeImpA = adenosine-5′-monophosphate-2-methylimidazolide; 2-MeImpD = diaminopurineriboside-5′-monophosphate-2-methylimidazolide. Oligomerizations were performed for 10 days at 0 °C using 10 pmol of template, 100 mM activated monomer, 100 mM $MgCl_2$, 1 M NaCl, and collidine·HCl buffer at pH 8.0.

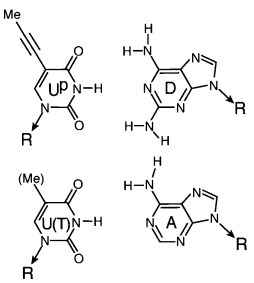


Fig. 1 Nucleobases with A·D·A/D·A(·D) hydrogen-bonding patterns used in the current study.

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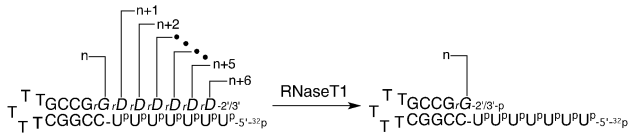
is consistent with formation of a 3′,5′-phosphodiester linkage between *n* and *n* + 1 (Schemes 1 and 2).

Success of the U^P template at directing incorporation of D (diaminopurine riboside-5′-phosphate) into a growing oligonucleotide strand may stem from several properties that propynyl groups are known to confer on nucleic acid helices. 5-Propynylation of successive pyrimidine nucleobases within the DNA component of a DNA–RNA helix confers both favorable long range cooperativity, and highly unusual stability to Watson–Crick base-pairs with three, rather than two, hydrogen-bonds.[9] We suggest that both of these features contribute in the present case. Non-enzymatic template-directed reactions of RNA proceed initially by ordering of multiple monomers on the template *via* non-covalent interactions, and subsequently by formation of covalent bonds between adjacent monomers after the reactive groups have been approximated.[10] Long range cooperative stacking by U^P residues in the template may contribute favorably to the initial multiple monomer association step. Additionally, the unusual stability of triply hydrogen-bonded base-pairs within propynylated DNA–RNA helices[9] may account for the overwhelmingly more favorable primer extension exhibited by D (3 W.–C. H-bond groups) over A (2 W.–C. H-bond groups) under the direction of template U^P.

Finally, we note that diaminopurine completely replaces adenine in the genetic material of some bacteriophage.[11] It is consequently clear that the purine component of the U^{P}D pair meets fitness criteria for integration into genetic material of a biological system. We are currently investigating the fitness of propynylated nucleobases in a variety of template-directed reactions with a view towards further reduction of sequence dependencies in non-enzymatic nucleic acid replication.



Scheme 2 Illustration of the ribonuclease T1 (RNase T1) assay used to determine the regiochemistry of the first phosphodiester bond formed during template-directed oligomerization according to Scheme 1 and Fig 2.

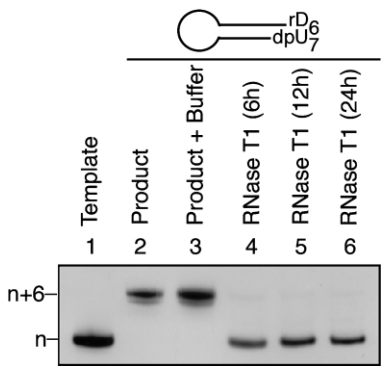


Fig. 3 Autoradiogram of 20% PAGE analysis of the RNase T1 assay of the oligomerization product from Fig. 2, lane 6, according to Scheme 2.

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Notes and references

† In response to a referee inquiry as to how 5-propynyluracil might be biosynthesized, we suggest the following pathway: electrophilic attack of propionaldehyde at the 5-position of uracil to provide 5-(propan-1-ol)uracil, elimination of water from the latter compound to give 5-(prop-1-ene)uracil, and then dehydrogenation[12] to yield 5-propynyluracil. We note that propionaldehyde is expected to have been a prebiotic reagent owing to its anticipated role in the formation by Strecker synthesis of α-amino-*n*-butyric acid, one of the most abundant amino acids produced in spark discharge experiments and found in carbonaceous chondrites.[13,14]

‡ Irrespective of their prebiotic plausibility, these compounds represent a particular example of a possible general solution to difficulties associated with abiotic replication of natural nucleotides–pyrimidines bearing 5-substituents that improve base-stacking and hydrogen-bonding characteristics. Additional examples, based on robust prebiotic reagents remain to be defined.

§ Templates were prepared on an ABI 391EP automated synthesizer using standard protocols and commercially available phosphoramidites. U^P phosphoramidite was purchased from Glen Research. Diaminopurineriboside was purchased from ChemGenes and phosphorylated using standard conditions.[15] Nucleoside 5'-phosphoimidazolides were prepared from the corresponding nucleoside phosphate according to the reported procedure.[16]

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