

Altering the Structure of DNA

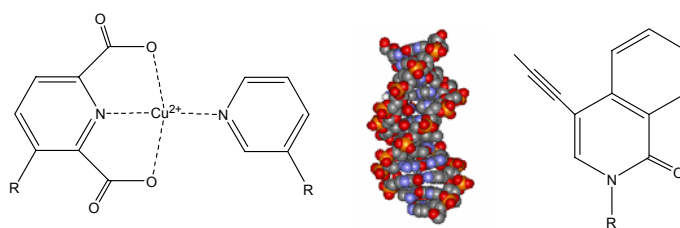
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ABSTRACT



The design of novel nucleotides has become an important aspect of nucleic acid chemistry. Several variations have been made on the natural nucleobases including nonpolar isosteres, hydrophobic bases, and metal coordinated base pairs. Modifications in the nucleobases of DNA can give rise to new functionality as well as probe the interactions of this important molecule with other biomolecules.

Deoxyribonucleic acid (DNA) is responsible for coding virtually every aspect of life. From a chemical standpoint DNA is a rather unreactive molecule, exerting its effects through noncovalent interactions with other nucleic acids and proteins. However, the structure of DNA can be modified in order to perturb its function. Modified nucleic acids have been widely exploited for a variety of reasons. For example, alterations have been made in order to study DNA-binding proteins,^{1,2} regulate gene expression,³ and even direct synthetic reactions.⁴

One area of active research on nucleic acid structure involves changing the structure of the nucleobases to determine the importance of Watson-Crick hydrogen bonds in duplex stability. To investigate this, a series of non-hydrogen bonding nucleoside analogues were synthesized (Figure 1). Difluorotoluene (dF) is a shape mimic of thymine (dT) in which fluorines replace carbonyl oxygens and nitrogens are substituted with sp²-hybridized carbons. This results in a nucleoside that is the same size as thymine

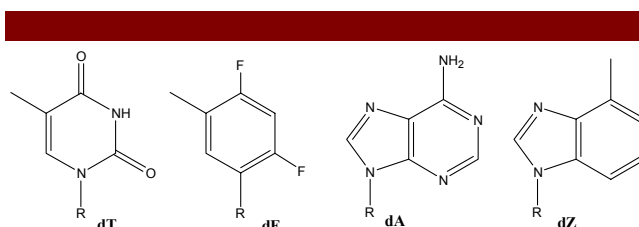


Figure 1. The structures of thymine and adenine and their nonpolar isosteres: difluorotoluene (dF) and 4-methylbenzimidazole (dZ), respectively. R: 2'-deoxyribose.

but lacks the ability to form hydrogen bonds.⁵ Replacement of dT with dF in an oligonucleotide opposite dA results in destabilization of the duplex by approximately 5 kcal/mol (Table 1). This destabilization can be attributed to the unfavorable enthalpic cost of desolvating adenine without the formation of stabilizing Watson-Crick hydrogen bonds. This also explains the observation that a dZ:dF base pair⁶ is 1.5 kcal/mol more

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(2)Morales, J. C.; Kool, E. T. *Nature Struct. Biol.* **1998**, *5*, 950-954.

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stable than either a dF:dA or dZ:dT base pair in the same sequence.^{5,7}

Table 1. Stability of Duplex DNA Containing Nonpolar Isosteres.

X	Y	T _m (°C) ^b	ΔG ₂₉₈ ^o (kcal)
dT	dA	39.4	12.3
dF	dA	21.4	7.4
dF	dT	20.2	7.3
dF	dF	28.6	8.9
dA	dT	39.8	12.4
dZ	dT	20.8	7.5
dZ	dF	30.3	8.9

^aData taken from ref. 6 and 8. ^bMeasured in the sequence d(CTTTTCTTCTT)-d(AAGAAAGAAAAG).

These nonpolar analogues also enabled researchers to probe the mechanism of polymerase fidelity. While size complementarity is acknowledged, the accuracy of DNA replication has been attributed to the ability of nucleobases to form complementary hydrogen bonds.⁸ The non-hydrogen bonding analogues are the first molecules that impair hydrogen bond formation without drastically affecting the size of the nucleobase. It was found that the inability to form hydrogen bonds does not diminish the fidelity of DNA polymerase (Figure 2).^{2,9,10} Thus, DNA replication is based solely on steric interactions between the template base, the incoming nucleotide triphosphate, and the enzyme's active site. The interested reader is referred to a number of excellent reviews on the implications this has on polymerase fidelity.¹¹⁻¹³ A better understanding of enzyme mechanisms provides direction for the development of more efficient methods for regulating enzyme activity. Therefore, this important discovery about the mechanism of DNA replication could open the door for innovative drug design targeting pathogenic DNA polymerases, such as HIV reverse transcriptase.

In addition to synthesizing nucleotides which model the natural nucleotides, novel nucleobases have been prepared that are useful in other applications. These include the use of fluorescent nucleotides to probe for DNA damage, as well as use in sensors and various materials.¹⁴⁻¹⁶

(6)While these analogues are not basic, they will be referred to as nucleobases.

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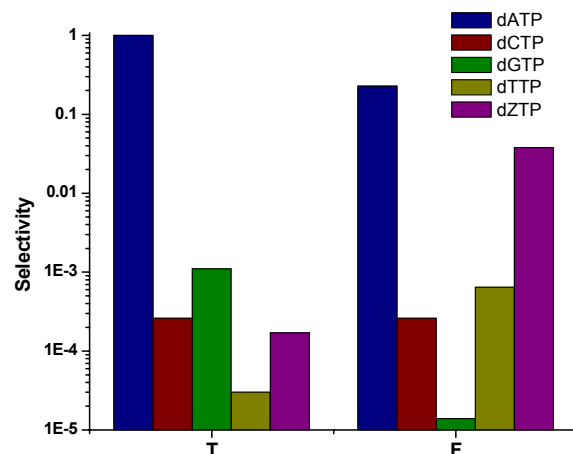


Figure 2. Selectivity of dNTP incorporation opposite thymine (T) or difluorotoluene (F) by the exonuclease deficient Klenow fragment. The efficiencies are normalized for dATP insertion opposite T. Data taken from ref. 2, 9, 10.

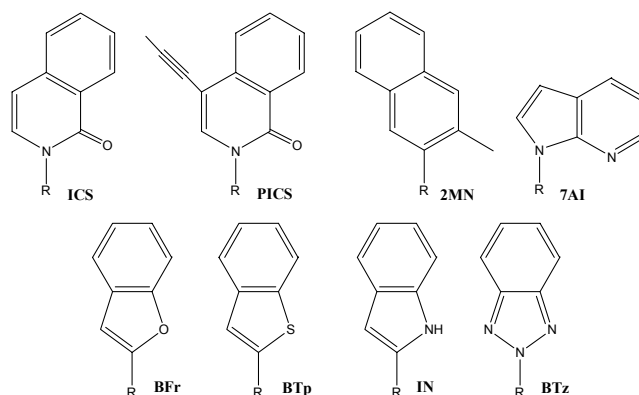


Figure 3. Unnatural nucleobases are being investigated for use in expanding the genetic code. These bases are incapable of forming Watson-Crick hydrogen bonds but stabilize duplex DNA through base stacking and hydrophobic interactions. R: 2'-deoxyribose.

Another ambitious area of research is to add a third base pair to the genetic code, thereby expanding the amount of information stored in DNA. Originally, effort was focused on pyrimidine- and purine-like nucleobases containing new arrangements of hydrogen bonds.¹⁷⁻¹⁹ However, given Kool's results on the ability of polymerase to replicate

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DNA in the absence of hydrogen bonds, hydrophobic bases have been investigated (Figure 3). While introduction of non-hydrogen bonding base pairs is often destabilizing to the DNA duplex,²⁰⁻²² the 7-propynyl isocarbostryl nucleoside, PICS, is able to form stable base pairs with itself. In fact, a PICS:PICS base pair is more stable than both a dA:dT and dC:dG base pair.^{23,24} Unfortunately the fidelity of polymerases replicating templates containing unnatural nucleobases is diminished. The hydrophobic bases prefer to self-pair over the formation of a mispair with a natural nucleotide by less than 60-fold (Figure 4).^{22-23,25} For comparison, incorporation of a correct versus incorrect nucleotide for native nucleobases is 10^4 - 10^5 . With such a low selectivity, the bases have limited use in expanding the genetic code.

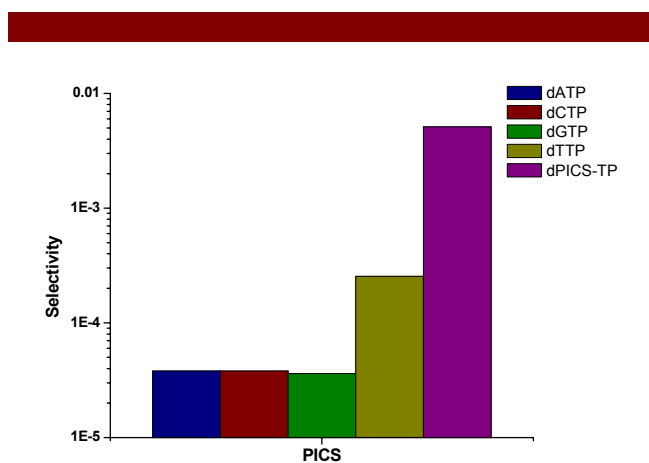


Figure 4. Selectivity of dNTP incorporation opposite 7-propynyl isocarbostryl nucleotide (PICS) by the exonuclease deficient Klenow fragment. The efficiency for nucleotide incorporation is compared to the insertion of dTTP opposite dA. Data taken from ref. 23.

Another significant problem in terms of replicating hydrophobic bases is the inability of polymerase to extend past the modified base pair. Even if the modified nucleobase is efficiently replicated by DNA polymerase, DNA synthesis is often stalled.^{26,27} Efficient extension of a primer is thought to be mediated by an interaction between a hydrogen bond acceptor located in the minor groove of

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the DNA duplex and the polymerase binding pocket. In order to probe these interactions, hydrophobic bases containing various hydrogen bond donor/acceptor groups in the minor groove have been developed (Figure 3).²⁶ Extension beyond a BTz self pair was the most efficient of the unnatural bases studied. However, it is still extended 200-fold less efficiently than past a natural base pair. It is believed that the imine functionality of BTz interacts with the polymerase binding pocket to create a more favorable orientation between the 3'-OH of the primer and the incoming nucleotide triphosphate than the other hydrophobic bases. To confirm this analysis, the ratio of the rate of incorporation of dNTP to α (S)dNTP was compared to determine the rate determining step of primer extension. In DNA replication, the rate determining step of primer extension varies depending on the base pair at the 3'-terminus of the primer. If a correct base pair is present, the rate determining step is a conformational change in the protein. However, when a mismatch is present, nucleophilic attack of the primer's 3'-OH to the α -phosphate of the incoming nucleotide triphosphate is rate limiting.²⁸ The rate determining step can be probed using α (S)dNTP, which inhibits nucleophilic attack on the α -phosphate. For extension past BTz, a $k_{cat}(dNTP)/k_{cat}(\alpha(S)dNTP)$ ratio of 34 was obtained, indicating that nucleophilic attack is rate determining.²⁶ Thus, even using the most efficiently extended hydrophobic base, the unnatural base pair perturbs the orientation of the 3'-OH. Further modifications of the nucleobase are needed in order to obtain the correct orientation of the primer's terminus.

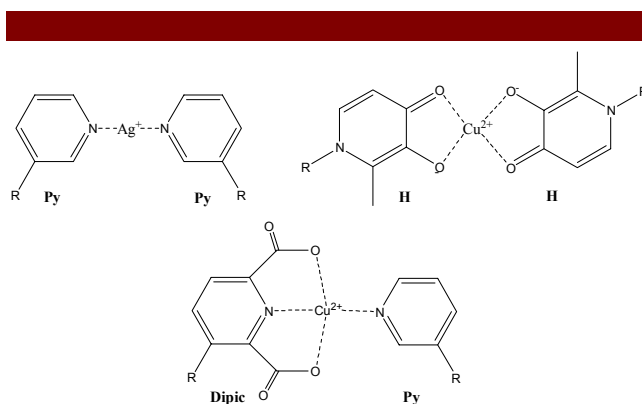


Figure 5. Metal coordinated base pairs are explored as stabilizing interactions in duplex DNA. These modified nucleobases also serve to arrange metal ions in solution, which could have value in novel materials. R: 2'-deoxyribose

Another area that has been explored is the use of a metal coordinated base pair to stabilize the helix. Instead of forming Watson-Crick hydrogen bonds, nucleobases have been prepared which form a base pair as ligands of a metal ion (Figure 5). Several metal coordinated base pairs have

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been synthesized to date.²⁹⁻³¹ The simplest of these is a base pair composed of two pyridines (Py) linked through a Ag(I) ion.³⁰ Addition of Ag(I) to a DNA duplex containing pyridine raises the T_m of the duplex by almost 7°C compared to the duplex in the absence of metal coordination (Table 2). While this stabilization is significant, the pyridine-Ag^I-pyridine base pair is still much weaker than a dA:dT base pair. To improve upon the stability of the duplex, bidentate nucleobases were designed. One of these, a Cu(II) mediated hydroxypyridone (dH) base pair has proven to be extremely stable.^{31,32} When incorporated into DNA in the presence of equimolar Cu²⁺, the T_m of the duplex increases almost 6°C over that of a duplex containing a dA:dT base pair.³¹ Thus, copper mediated base pair formation, increases the T_m approximately 13°C. Furthermore, a stretch of 5 dH-Cu-dH base pairs have been successfully incorporated into duplex DNA. The presence of multiple modified bases does not affect the structure of the duplex. Upon addition of Cu²⁺ to the oligonucleotide, duplex DNA is observed with a typical right-handed helix.³² These nucleobases could be of interest both for expanding the genetic code and for use in nanomaterials such as molecular magnets or wires.²⁹⁻³²

Table 2. Stability of Duplex DNA Containing Metal Coordinated Base Pairs.

X:Y	Metal Ion ^a	T_m (°C)
A:T	None	46.5 ^b
A:T	3 mol Ag ⁺	47.7
Py:Py	None	34.2
Py:Py	1 mol Ag ⁺	38.0
Py:Py	3 mol Ag ⁺	41.0
A:T	none	44.2 ^c
A:T	1 mol Cu ²⁺	44.2
H:H	None	37.0
H:H	1 mol Cu ²⁺	50.1
A:T	15 mol Cu ²⁺	41.1 ^c
Py:Dipic	None	No transition
Py:Dipic	1 mol Cu ²⁺	38.6
Py:Dipic	5 mol Cu ²⁺	40.1

^aMolar equivalents of metal ion to DNA duplex. ^bMeasured in the sequence d(AAAAAAAAAAXAAAAAAAAA)-d(TTTTTTTTTTYYYYTTTTTTTT). Values taken from ref. 30. ^cMeasured in the sequence d(CACATTAXTGGTGTG)-d(TACAACAYTAATGTG). Values taken from ref. 29,31. Py: Pyridine; H: Hydroxypyridone; Dipic: Pyridine-2,6-dicarboxylate.

In anticipation of replication by DNA polymerase, nucleobases were designed in which one base would

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chelate the metal ion with higher affinity over the complementary strand. Pyridine-2,6-dicarboxylate nucleobase (Dipic) and a pyridine nucleobase (Py) coordinate Cu²⁺ via a [3+1] square planar geometry.²⁹ In addition to keeping the metal ion centered on Dipic over Py, this arrangement of ligands should discourage Cu²⁺ association with the natural nucleobases. While Dipic and Py destabilize the helix in the absence of transition metal ions, addition of Cu²⁺ results in a base pair with stability comparable to a dA:dT base pair.²⁹ This stability is not surprising since a metal ligand bond can have a bond dissociation energy as low as 10 kcal/mol which is similar to the 2-3 hydrogen bonds holding natural nucleobases together.

Further studies are required to determine if DNA polymerases can tolerate such an unusual base pair. The incorporation of metal coordinated nucleotides into DNA opposite native nucleotides has been examined. A series of phenyl triphosphates containing 0-2 phenolic hydroxyl groups were found to inhibit DNA synthesis.³³ However, studies were not conducted to determine the ability of polymerase to incorporate the modified triphosphates opposite metal coordinated nucleotides. If this process occurs efficiently, these nucleotides could be extremely selective during DNA replication and thus, a prime candidate for the expansion of the genetic code.

Unnatural nucleobases have proven to be extremely interesting in terms of their effects on the properties of DNA. The design of novel base pairs is being exploited to add functionality to the nucleic acid and is only limited by the creativity of the chemist. Future work will likely use nucleobase analogues to elucidate the mechanisms of DNA binding proteins, inhibit DNA polymerases in an anti-neoplastic or anti-viral context, and for the design of novel nanomaterials.^{11,32,33}

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