around an inversion center with occupancies of 0.50. The carbon atom of one coordinated methanol molecule is disordered over two sites (0.74:0.26). The carbon atom of the methoxide ion is disordered over three sites (0.38:0.31:0.31). The asymmetric unit is occupied by one methanol solvent molecule and four partially occupied and one disordered water molecule (0.68:0.32). The hydrogen atoms of the hydroxyl groups and the methoxide ion could not be located in the difference fourier synthesis.[14]

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[14] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC-100739 (**1**) and 100744 (**2**, **3**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: int. code +(44)1223 336-033; e-mail: deposit@ccdc.cam.ac.uk). Red-green stereo presentations of **1**–**3** are available on the WWW under http://www.organik.uni-erlangen.de/saalfrank/index.html.

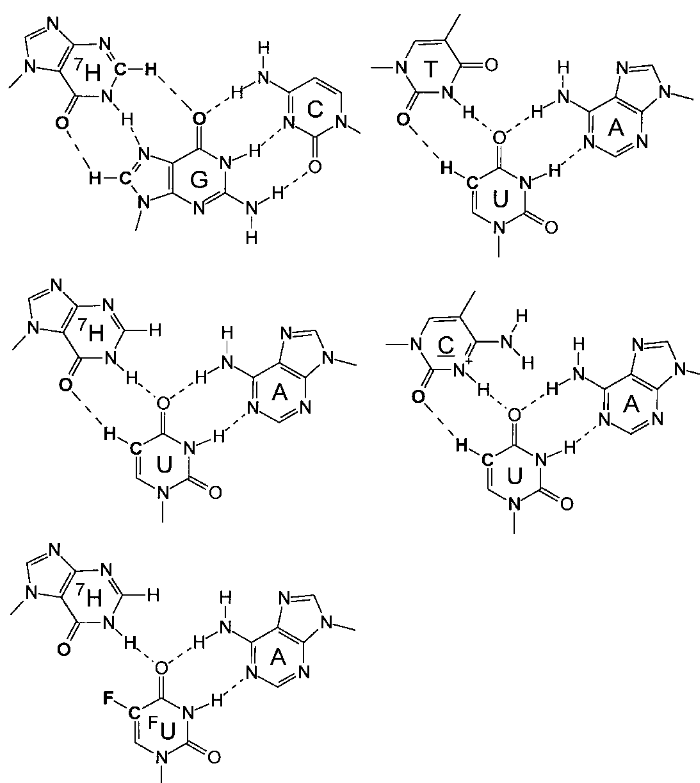


Figure 1. Experimentally investigated base triples in the parallel triple-helical binding motif.

Evidence for C–H ··· O Hydrogen Bond Assisted Recognition of a Pyrimidine Base in the Parallel DNA Triple-Helical Motif**

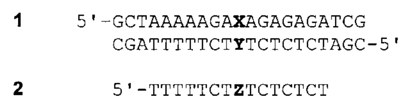
Judith Marfurt and Christian Leumann*

The structural and energetic influence of nonconventional C–H ··· O hydrogen bonds on molecular recognition has become a focus of interest. Whereas a large body of structural data evidences their importance in molecular recognition of small molecules in the crystalline state,[1] only limited experimental data is available on their contribution to biomolecular recognition in solution.[2] C–H ··· O hydrogen bonds have only recently begun to be used as design elements for directing intermolecular organization of small organic molecules (crystal engineering).[1, 3] Their use as a designer element for biomolecular complexation is virtually unexplored.

We recently reported on the selective recognition of G–C base pairs in DNA duplexes by parallel complementary oligodeoxynucleotides containing the unnatural nucleoside 7-(2′-deoxy-β-D-ribofuranosyl)hypoxanthine (^{7}H).[4] Although only one conventional N–H ··· N hydrogen bond between ^{7}H and G can be formed, the ^{7}H · G–C base triple (Figure 1) has the same stability as the canonical C̲$^+$ · G–C base triple (C̲ = 5-methyl deoxycytidine) at pH 7.0, in which the base of the third strand is bound through two conventional hydrogen bonds to the purine base of the Watson–Crick duplex. Since precise structural data were lacking, we empirically attributed the extra stability to the presence of one or two C–H ··· O hydrogen bonds flanking the conventional hydrogen bond of the third-strand base.

To explore whether such weak electrostatic interactions can be further exploited for the recognition of DNA duplexes by oligonucleotides, we investigated the recognition of pyrimidine bases by considering such C–H ··· O hydrogen bonds. Specifically we investigated the interaction of a deoxyuridine unit in a DNA duplex with a ^{7}H unit in the third strand (Figure 1).

The DNA target duplexes **1** as well as the third-strand oligonucletides **2** (Figure 2) were synthesized by standard phosphoramidite chemistry from commercially available and recently described DNA building blocks.[4] The binding efficiency of the third strands to the target duplex was



X	G	dU	d^{F}U	T
Z / **Y**	C	A	A	A
C̲	37.7 (-3.0)	24.2 (0)	18.5 (2.1)	17.9 (1.1)
^{7}H	40.0 (-3.4)	30.0 (-0.8)	24.0 (0.3)	18.2 (1.7)
T	---	27.7 (-0.7)	19.5 (1.9)	16.9 (2.0)

Figure 2. Top: Sequences of the target duplexes **1** and third strands **2**; bottom: T_m values [°C] for third-strand dissociation from UV melting curves (λ = 260 nm) and (in parentheses) ΔG values [kcal mol^{-1}] for triplex formation at 25 °C. Total single-strand concentration: 1.6 µM. Buffer: 10 mM sodium cacodylate, 100 mM NaCl, 0.25 mM spermine, pH 7.0.

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assessed by an analysis of the UV melting curve in the buffers specified.[5] The melting temperatures (T_m) for dissociation of the third strand as well as the calculated Gibbs energies for triplex formation at 25 °C (ΔG) extracted from the melting curves[6] are summarized in Figure 2. A representative collection of melting curves are depicted in Figure 3.

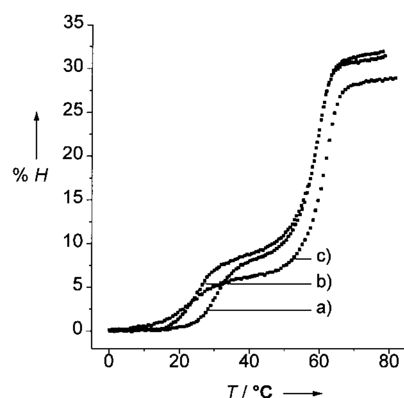

Figure 3. UV melting curves (% H = relative hyperchromicity, λ = 260 nm). a) **1·2** (**Z·X–Y** = ^{7}H · dU–A; b) **Z·X–Y** = ^{7}H · d^{F}U–A; c) **3·4**. Oligonucleotide concentration and buffer as in Figure 2 with the exception of pH 6.0 for c).

Inspection of the T_m data reveals that the oligonucleotide **2** (**Z** = ^{7}H) efficiently binds to the target duplex in which the base uracil is opposite to the ^{7}H residue (**1**; **X**, **Y** = dU, A) with formation of a ^{7}H · U–A base triple. This triplex is moderately less stable than the standard oligopurine/oligopyrimidine reference triplex displaying the conventional **Z·X–Y** = $\underline{C}^+$ · G–C base triple ($\Delta T_m = -7.7$ K). If the ^{7}H residue of the third strand is placed opposite to a thymine unit (**Z·X–Y** = ^{7}H · T–A), a strong destabilization of the triplex relative to that containing the ^{7}H · U–A base triple is observed ($\Delta T_m = -11.8$ K). This corresponds to a typical mismatch situation and is due to the 5-methyl group of thymine, which completely obstructs the base-recognition process for steric reasons.

To investigate a possible energetic contribution of the C–H ··· O hydrogen bond to triplex stability, we replaced the 2′-deoxyuridine in the target duplex by 5-fluoro-2′-deoxyuridine (d^{F}U). The fluorine atom represents the closest non-isotopic isostere of hydrogen with an opposite electrostatic environment. As expected, this base is recognized by ^{7}H with distinctly lower affinity than uracil (ΔT_m of the corresponding triplexes = -6.0 K). This difference in T_m translates to a difference in binding enthalpy ($\Delta\Delta G$) of about -1.1 kcal mol^{-1} at 25 °C.

A similar hydrogen-bonding pattern between **X** and **Z** appears if ^{7}H in the third strand is replaced by either thymidine or 5-methyl-deoxycytidine (Figure 1, right). Indeed, replacement of ^{7}H by thymidine leads to a decrease in T_m of only 2.3 K. Thus, both nucleosides display almost equal efficiency in uracil recognition. Again, positioning the base thymine opposite to d^{F}U leads to a strong decrease in binding efficiency of the corresponding third strand ($\Delta T_m = -8.3$ K). Offering a thymidine unit in the duplex as the complexing partner for thymidine in the third strand results in a mismatch. Similar results were obtained for third strands containing 5-methyl-deoxycytidine, although on a lower affinity level. The overall lower T_m values most probably arise from the need for protonation of the base, an unfavorable process at pH 7.0.

We calculated the heat of formation (ΔH_f^0) of the base pairs **A** and **B** (Figure 4) in the gas phase with semi-empirical methods. Base pair **A** corresponds to the arrangement **Z·X** = ^{7}H · dU (Figure 2), whereas **B** represents an isomeric base pair

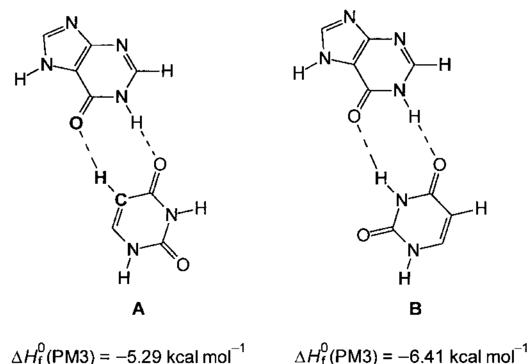

Figure 4. Structures and standard heats of formation (ΔH_f^0) of base pairs **A** and **B** calculated with the semiempirical model PM3 for the gas phase as implemented in the program package Spartan V4.0 (Wavefunction Inc., USA).

between ^{7}H and uracil that is connected through two conventional hydrogen bonds. Interestingly, the heat of formation (ΔH_f^0) of base pair **A** displaying one conventional and one C–H ··· O hydrogen bond is only 1.12 kcal mol^{-1} less exothermic than **B**, which has two conventional hydrogen bonds. Thus, replacement of a conventional hydrogen bond by a C–H ··· O bridge is compromised by a loss of only about 1 kcal mol^{-1} in binding enthalpy. This is reasonably well reflected in the experimentally determined difference in stability between the triplex displaying a $\underline{C}^+$ · G–C base triple and that displaying the ^{7}H · U–A base triple ($\Delta\Delta G \approx$ 2 kcal mol^{-1} at 25 °C). For comparison, typical $\Delta\Delta G$ values for the triplex containing $\underline{C}^+$ · G–C and a true mismatch system are about 5 kcal mol^{-1} (e.g., T · T-A, Figure 2).

To explore the limitations of the C–H ··· O hydrogen bond assisted recognition of uracil with ^{7}H, we investigated the formation of the triple helix **3·4** (Figure 5), which is designed

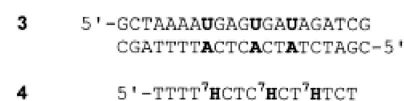

Figure 5. Sequence of the target duplex **3** and third strand **4**.

to incorporate three ^{7}H · dU interactions. This corresponds to a sequence context with a pyrimidine content of 20 % in the triplex-recogniton region of the underlying duplex. As shown in Figure 3, binding at pH 6.0 is maintained even in this system with a T_m of 23.6 °C for third-strand dissociation. As expected, third-strand binding is less efficient in this case ($T_m \approx 1.5$ °C at pH 7.0) than for an all-purine target sequence under comparable conditions. We anticipate that in such systems the loss of affinity might be compensated by combining C–H ··· O hydrogen bond assisted recognition of pyrimidine bases with

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further modifications to enhance triplex stability, for example replacing C̲ by the 2-aminopyridine nucleoside **P** for recognition of guanine.[9, 10]

We can not definitely rule out the possibility of an energy-neutral contribution from the C–H···O arrangement in the corresponding base triples. Nevertheless, the experimental results described here are in good agreement with the general view that the energetic benefit of forming the C–H···O hydrogen bond is made up of a small, positive, direct electrostatic contribution[1] and a positive contribution due to the alleviation of a destabilizing interaction that arises if a hydrogen-bond acceptor (such as a carbonyl oxygen atom) is inactive or involved in secondary hydrogen bonding, for example, to solvent.[11] In any case, the final proof for the existence of a C–H···O hydrogen bond in the triplexes discussed here has to come from high-resolution structure analysis.

The efficient complexation of uracil by ^{7}H or T presented here is of direct relevance to the molecular recognition and folding of RNA. C–H···O hydrogen bonds in nucleic acids can compete with conventional hydrogen bonds under certain circumstances. This was shown by a recent X-ray structure analysis of an RNA duplex in which a U·U base pair built from one conventional and one C–H···O hydrogen bond was preferentially formed over an isomorphous base pair with two conventional hydrogen bonds.[12]

In applying this concept to triple-helical DNA recognition, the following restrictions have to be considered: 1. Uracil is not a DNA base, and there is no clear way to use the presented concept of C–H···O hydrogen bond assisted pyrimidine recognition for the complexation of the natural DNA base thymine. 2. Both thymidine and ^{7}H, as components of a third strand, have a primary binding preference for the purine bases adenine and guanine, respectively, which compromises the selectivity in the formation of DNA triple helices. This issue of selectivity, however, might be addressable by base design.

We have shown here that unconventional C–H···O hydrogen bonds may be advantageously used as a design element for enhancing the efficiency of weak base–base interactions in the complexation of nucleic acids. We are currently trying to expand this concept to triplex-mediated recognition of cytosine. Efficient and selective pyrimidine recognition definitely constitutes one way of extending the sequence range of triplex formation in nucleic acids, a widely sought but hitherto unsolved goal.[13]

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Global and Local Aromaticity in Porphyrins: An Analysis Based on Molecular Geometries and Nucleus-Independent Chemical Shifts**

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Dedicated to Professor Emanuel Vogel on the occasion of his 70th birthday

How and to what extent does aromaticity contribute to the geometric and electronic structure of porphyrins? Despite its widespread use for nearly two centuries, "aromaticity" is difficult to define.[1] Usually, this is done on the basis of energetic, geometric and magnetic criteria.[2] Molecular geometries are often the most accessible, and aromaticity indices have been derived from bond lengths.[3] Recently the nucleus-independent chemical shift (NICS) was proposed as a new magnetic criterion, based on MO calculations of the magnetic shieldings at the centers or other points of aromatic systems.[4] The aim of this paper is to deduce the electronic structure of porphyrins based both on a large number of geometries retrieved from the Cambridge Structural Database [5] and on computed NICS values.

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