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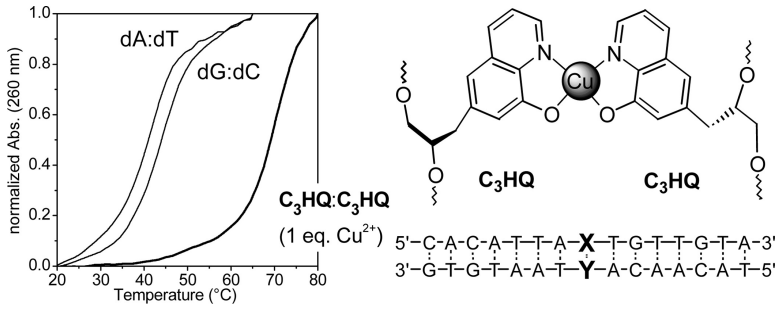

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An Extremely Stable and Orthogonal DNA Base Pair with a Simplified Three-Carbon Backbone

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DNA plays an increasingly important role in bioorganic chemistry, biotechnology, and material science due to its ingeniously simple complementary base-pairing rule: A pairs with T, and G with C. It is apparent that modifying the chemical and physical properties of DNA without affecting the complementary base pairing will have a tremendous impact on future applications. For example, increasing the electrical conductivity of DNA by chemical modification of the base pairs may allow for the self-assembly of complex molecular-scale electrical devices.[1]

We started out with the goal to design functional unnatural nucleotides that are structurally simplified and thus much easier to access in large quantities, while retaining the desired base-pairing properties. For example, a simple acyclic DNA-like backbone would reduce the synthetic complexity dramatically. However, Schneider and Benner demonstrated more than a decade ago, that even a single flexible acyclic nucleotide in DNA already leads to a strong destabilization of the duplex.[2] We envisioned that it may still be possible to use a simplified backbone by overcompensating the potential loss of preorganization with interstrand base-pairing strength. We here present a surprising outcome of this design strategy which resulted in an exceptionally stable and orthogonal artificial base pair having a minimal acyclic three-carbon backbone.

First, we designed a base-pairing scheme with superior stability. Base-pairing schemes with alternate H-bonding,[3] pairing through hydrophobic packing,[4] and metal coordination-driven base pairing[5] have been developed. We decided to create a DNA base pair with superior stability by combining hydrophobic forces and strong metal coordination in one base pair as shown in Figure 1. We chose 8-hydroxyquinoline as a promising candidate because it has an extended hydrophobic aromatic surface, ideal for undergoing hydrophobic stacking in DNA, in addition to being an exceptionally strong bidentate ligand for a variety of transition metal ions.[6]

The nucleotide **HQ** containing a regular 2′-deoxyribose backbone (see Figure 1) was synthesized following a lengthy standard route (13 steps in the longest linear sequence plus one diastereomer separation) and incorporated in the middle of a 15mer deoxyoligonucleotide as shown in Figure 2. In the absence of any transition metal ions, a 1:1 mixture of complementary 15mer oligonucleotides containing the **HQ**:**HQ** homopair displays a melting temperature (T_M) of 36.1 °C, as determined by UV-monitored thermal denaturation. For comparison, the duplexes containing a dA:dT or dG:dC instead of **HQ**:**HQ** melt at 41.3 °C and 44.6 °C, respectively. Mismatches between natural bases show stabilities of 31 °C (dG:dT) or less under our experimental conditions. Thus, **HQ**:**HQ** forms quite stable base pairing even in absence of any transition metal ions. This effect can be attributed to the high hydrophobicity of **HQ**.

Upon the addition of just one equivalent of Cu^{2+}, the melting point increases by 29 °C reaching a T_M of 65 °C. The T_M of **HQ**:**HQ** in the presence of Cu^{2+} is more than 20 °C higher compared to the natural base pairs dA:dT and dG:dC (Figure 2A). To the best of our knowledge, a base pair with such strong interstrand pairing properties is unprecedented. It can be expected that in this

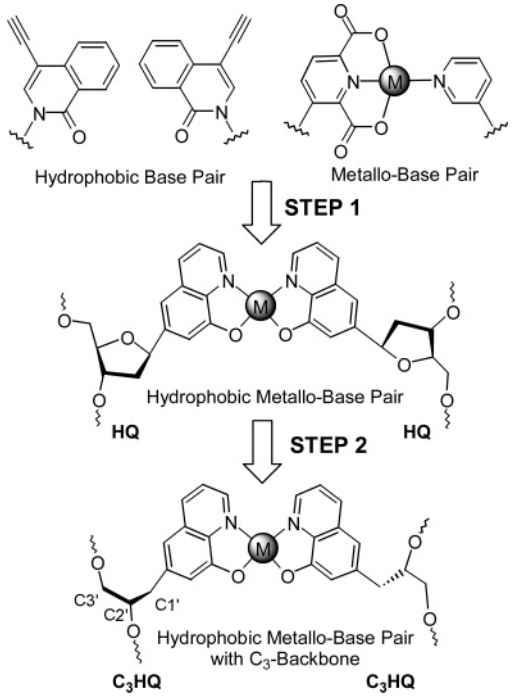

Figure 1. Design rationale for a simplified completely artifical base pair in DNA.

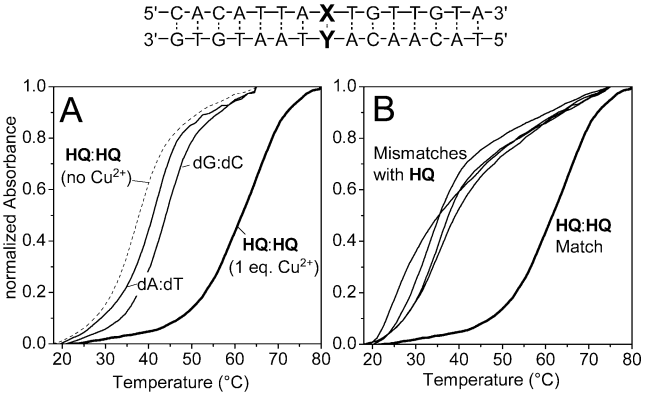

Figure 2. UV-melting curves of duplex deoxyoligonucleotides with different base pairs at position **X**:**Y**. (A) **HQ**:**HQ** (T_M = 36.1 °C without Cu^{2+}, T_M = 65 °C with 1 equiv of Cu^{2+}), dA:dT (T_M = 41.3 °C), dG:dC (T_M = 44.6 °C). (B) Melting curves of mismatches with **HQ** in the presence of 1 equiv of Cu^{2+}. **HQ**:T (T_M < 30 °C), **HQ**:C (T_M = 32.5 °C), **HQ**:A (T_M = 34.7 °C), **HQ**:G (T_M = 35.4 °C). The hyperchromicity was in all cases 15−24%. Experiments were performed in 10 mM sodium phosphate, pH 7.0, 50 mM $NaClO_4$, with 2 μM of each single strand, and under argon atmosphere to prevent photooxidation of **HQ**. $Cu(NO_3)_2$ was used as the source for Cu^{2+}.

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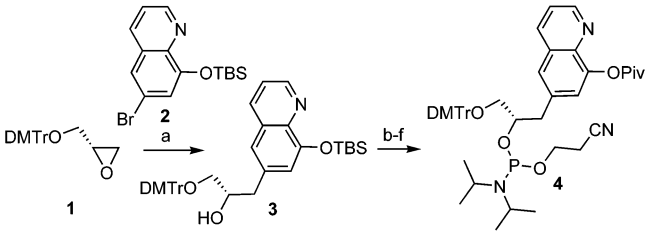

Figure 3. Synthesis of the C_3-nucleotide **4** for the automated nucleic acid synthesis. (a) First, addition of *sec*-BuLi (THF, −78 °C) to **2**, followed by $MgBr_2$ (in situ prepared from $BrCH_2CH_2Br$ and Mg), and cat. CuI, followed by the addition of epoxide **1** (69%). (b) TBSCl, DMAP, imidazole. (c) Cs_2CO_3. (d) *t*BuCOCl, DMAP. (e) TBAF (f) $(iPr_2N)(OCH_2CH_2CN)PCl$, $(iPr)_2EtN$ (49% over steps b−f).

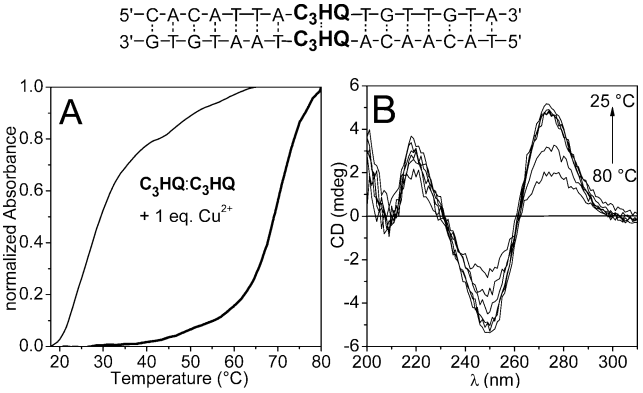

Figure 4. (A) UV-melting curves of the shown duplex above (2 μM each strand) containing the acyclic nucleobase pair **C_3HQ**:**C_3HQ** without and with one equivalent of Cu^{2+} (T_M = 70.5 °C). The hyperchromicity is 16% and 18%, respectively. (B) CD-spectra of the same duplex (10 μM each strand) in the presence of 1 equiv of Cu^{2+} at 80, 70, 60, 50, 40, and 25 °C. Experiments were performed in 10 mM sodium phosphate, pH 7.0, 50 mM $NaClO_4$, and under argon atmosphere. $Cu(NO_3)_2$ was used as the source for Cu^{2+}.

base pair the two 8-hydroxyquinoline ligands coordinate a central Cu^{2+} ion in an approximately square planar fashion as indicated in Figure 1.[5b]

To test the pairing specificity of the Cu^{2+}-dependent **HQ**:**HQ** base pair, we measured the T_M's of all mismatches with the natural strands. The melting curves are shown in Figure 2B. Compared to **HQ**:**HQ**(+Cu^{2+}) the mispairs with natural bases lead to a strong decrease in melting temperatures of more than 30 °C. Thus, the base pair **HQ**:**HQ**(+Cu^{2+}) shows exceptionally strong base-pairing strength and orthogonality and is therefore a promising candidate to reduce the complexity of the backbone in the next step.

We chose the three-carbon derivative **C_3HQ** as shown in Figures 1 and 3. This backbone is derived from Eschenmoser's L-α-threofuranosyl nucleoside[7] by eliminating a CH_2O unit from the tetrahydrofuran ring, and we envisioned that this scaffold is economically accessible by ring opening of "spring-loaded" epoxides.[8]

Accordingly, inexpensive commercially available *S*-(−)-glycidol was tritylated to **1** and the epoxide regioselectively ring-opened with metalated **2** to yield **3** in 69% yield (Figure 3). Exchange of the protection group at the 8-hydroxyquinoline followed by introduction of a phosphoramidite yielded the building block **4** for the automated oligonucleotide synthesis. This procedure is short and simple and does not require any separation of isomers.

We next investigated the stability of this new homopair **C_3HQ**:**C_3HQ** in duplex DNA. Without Cu^{2+}, no stable duplex formation is observed (Figure 4A). However, upon the addition of just one equivalent of Cu^{2+}, **C_3HQ**:**C_3HQ** gives cooperative UV-melting with a T_M of 70.5 °C. The UV-melting experiments are in agreement with CD measurements, which demonstrate a temperature-dependent melting of a B-form duplex (Figure 4B). It is very surprising that the stability of the simplified base pair **C_3HQ**:**C_3HQ**(+Cu^{2+}) surpasses that of **HQ**:**HQ**(+Cu^{2+}) ($\Delta T_M = +5.5$ °C). This is even more remarkable since the C_3-backbone is strongly destabilizing for the natural A:T base pair (T_M < 30 °C). We hypothesize that the expanded C1′-C1′ distance in the 8-hydroxyquinoline base pair can be accommodated with less strain in the slimmer acyclic backbone. It is also noteworthy that no stable base pairing is observed between **C_3HQ** and the natural deoxynucleotides (T_M's < 25 °C, 1 equiv of Cu^{2+}).

In summary, we have introduced a strategy for the design of a simplified artificial base pair. The nucleotide **C_3HQ** with a minimal three-carbon backbone displays unprecedented pairing strength and orthogonality in a homopair **C_3HQ**:**C_3HQ** in the presence of one equivalent of Cu^{2+}. It is quite a surprise that the pairing stability and selectivity even exceeds those of the related base pair **HQ**:**HQ**, having the regular deoxyribose backbone. This discovery of a synergy between an artificial backbone and base-pairing scheme opens new avenues for the economical design of modified oligonucleotides with tailored properties.

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Supporting Information Available: Experimental procedures for the synthesis of **HQ**, **C_3HQ** and their incorporation into DNA. This material is available free of charge via the Internet at http://pubs.acs.org.

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