deactivated fused silica column coated with dimethylpolysiloxane (1 m × 0.25 mm internal diameter, 0.002 μm film thickness). The reactor column was quickly heated to the temperature T and enantiomerization commenced. After the reaction time t the reactor column was rapidly cooled with liquid nitrogen and the new enantiomeric mixture was transferred onto the second separation column coated with Chirasil-β-Dex (12.5 m × 0.25 mm internal diameter, 0.4 μm film thickness, 110 °C), where the enantiomers were separated. The carrier gas used was helium. The experiment was repeated three times at each temperature. The rate constant k for inversion was calculated according to Equation (1) using the observed enantiomeric ratio (% *er*) of the major peak area, the temperature T, and the reaction time t.

$$k = \frac{1}{2t} \ln \frac{er+1}{er-1} \quad (1)$$

The mean values of $\ln(k/T)$ were plotted as a function of T^{-1} according to the Eyring equation. The activation parameters $\Delta H^{\neq}_{gas}$ and $\Delta S^{\neq}_{gas}$ were obtained from the gradient and the y intercept, respectively, of a linear fit. A statistical factor κ of 0.5 for a reversible inversion process was applied.

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Stable and Selective Hybridization of Oligonucleotides with Unnatural Hydrophobic Bases**

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DNA duplex stability and sequence specificity are based on the complementary Watson–Crick hydrogen-bonding (H-bonding) patterns of adenine with thymine (dA:dT base pair) and cytosine with guanine (dC:dG base pair). Additional, stable and selective base pairs would facilitate hybridization or encoding experiments in cases where natural sequences cross hybridize, or where increased information storage is desirable.[1–4] Moreover, the characterization of DNA containing such base pairs may provide increased insight into DNA structure and function.[5–15] We recently reported the synthesis and characterization of several unnatural nucleosides that possess hydrophobic groups instead of a purine or pyrimidine base.[2, 3] Among these, hydrophobic nucleosides containing the isocarbostyril core were particularly interesting due to their ability to form stable self-pairs in duplex DNA (Isocarbostyril = 1-hydroxyisoquinoline).[3] We now describe a series of isocarbostyril derivatives that culminate in a self-pair which is both more stable and more selective than either a dA:dT or a dC:dG pair in the same sequence context.

The isocarbostyril core was derivatized with a propynyl group at C7, a substitution known to impart increased stability to both natural[9, 16] and unnatural DNA,[2, 3] as well as with a C3 methyl group, which is expected to pack in the hydrophobic base interface. The 7-propynyl-3-methylisocarbostyril nucleoside (**PIM**) was synthesized and converted to phosphoramidites **5** as shown in Scheme 1.[17] The free base **2** was generated according to the procedure of Hirato et al.,[18] and

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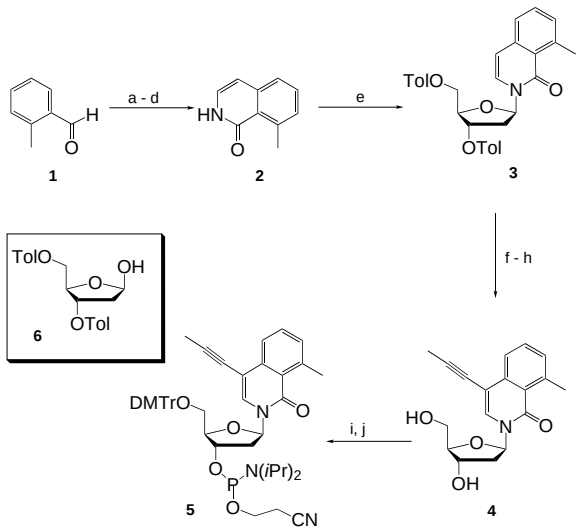


Scheme 1. a) $(MeO)_2CHCH_2NH_2$, benzene; b) Cl_3CCO_2Et, THF, $P(OMe)_3$, $TiCl_4$, CH_2Cl_2; c) H_2O_2, AcOH; d) Ac_2O, then NaOH; e) NaH, CH_3CN, **6**; f) ICl, CH_2Cl_2; g) $HC{\equiv}CCH_3$, CuI, $[PdCl_2(PPh_3)_2]$, NEt_3; h) NaOMe, MeOH; i) DMTrCl, pyridine; j) $(iPr)_2NP$-$ClOCH_2CH_2CN$, DIPEA, CH_2Cl_2. Tol = tolyl, DMTr = 4,4-dimethoxytriphenylmethyl, DIPEA = diisopropylethylamine.

the propynyl group was introduced by an iodination and a Sonogashira-coupling[19, 20] with the protected nucleoside **3**.[21] The isocarbostyril (**ICS**), 7-propynylisocarbostyril (**PICS**), and 3-methylisocarbostyril (**MICS**) nucleosides and phosphoramidites were synthesized as described previously.[2, 3] The thermal stability of the unnatural base pairs was evaluated by determining the melting temperature (T_m) of duplex **7** with X and Y = dA, dT, dG, dC, **ICS**, **PICS**, **MICS**, or **PIM** (10 mM 1,4-piperazinediethanesulfonic acid (PIPES), 100 mM NaCl, pH 7). The stabilities of native and unnatural base pairs are reported in Table 1.

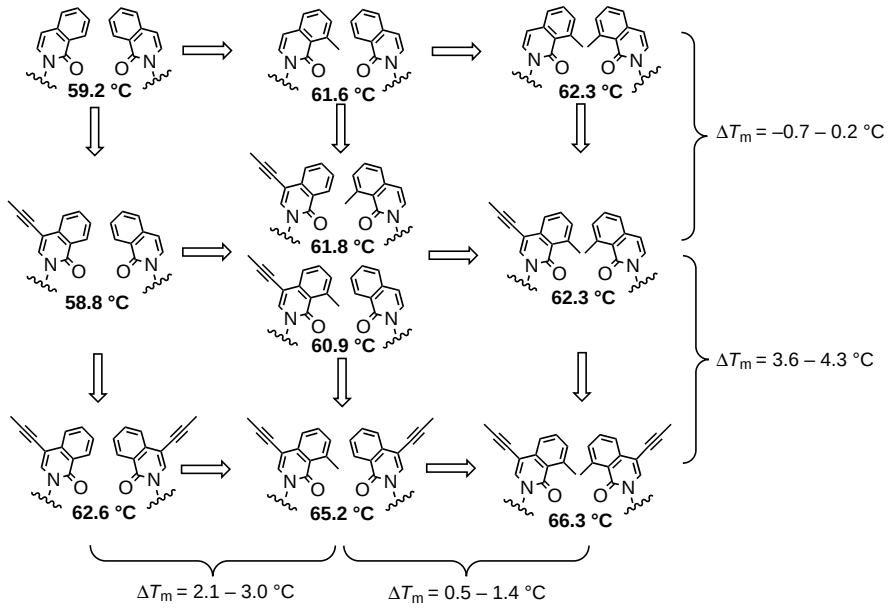
GCGTAC***X***CATGCG

CGCATG***Y***GTACGC

7

In the sequence context of duplex **7**, the least stable unnatural self-pair, **ICS**:**ICS** is as stable as a dA:dT pair (59.3 °C and 59.2 °C for **ICS**:**ICS** and dA:dT, respectively), as reported previously.[2] The relationship between structural modifications and thermal stability is illustrated in Scheme 2. Addition of a C3 methyl group to a single nucleobase, or to both nucleobases of a pair stabilizes the resulting base pair by 2.1 – 3.0 °C and 3.1 – 3.7 °C, respectively. The addition of a propynyl group to one nucleobase of the unnatural pair results in either little effect (for example, **MICS**:**MICS** versus **PIM**:**MICS**: $\Delta T_m = 0.2$ °C) or a destabilizing effect on duplex formation (as in **ICS**:**ICS** versus **PICS**:**ICS** and **MICS**:**ICS** versus **PIM**:**ICS**: $\Delta T_m = -0.7$ °C). However, the addition of a propynyl group to the both nucleobases of a pair results in a significant increase in duplex stability by 3.4 – 4.0 °C.

The additive effects of both methyl and propynyl substitution result in a remarkably stable **PIM**:**PIM** self-pair. The **PIM**:**PIM** self-pair is significantly more stable than a dC:dG base pair ($T_m = 66.3$ °C and 61.8 °C for **PIM**:**PIM** and dC:dG, respectively) in this sequence context. Furthermore, the combinations of methyl and propynyl substitution allow nucleobase combinations which result in base pairs with a range of stabilities, from the stability of a dA:dT pair to significantly more stable than a dC:dG pair.



Scheme 2. The relationship between structural modification and thermal stability.

Table 1. Denaturation temperatures (T_m) for oligonucleotide **7** containing a series of unnatural and natural base pairs (X:Y).[a]

X = Y =	**ICS**	**PICS**	**MICS**	**PIM**	A	T	G	C
ICS	59.3							
PICS	58.8	62.6						
MICS	61.6	61.8	62.3					
PIM	60.9	65.2	62.3	66.3				
A	55.1	55.5	53.6	55.1	52.7	58.7	52.3	48.8
T	53.0	53.7	55.1	54.7	59.2	49.8	52.8	47.2
G	52.2	51.4	54.8	56.2	55.4	53.3	50.5	61.8
C	51.0	54.5	54.8	53.4	48.4	45.0	60.7	44.8

[a] The melting temperature experiments were carried out on a Cary 300 - Bio UV/Vis spectrophotometer. The heating rates were 0.5 °C min^{-1}. Conditions: 3 μM DNA, 10 mM PIPES, 10 mM MgCl, 100 mM NaCl, pH 7.

Minimization of cross-hybridization, which results from mispairing within hybridizing oligonucleotides, is of great practical importance.[15, 22–27] To minimize nonselective hybridization with an expanded set of DNA bases, the unnatural bases must selectively pair with each other relative to mispairing with native bases. The selectivities of the unnatural bases were determined by synthesizing and characterizing a series of oligonucleotides containing mispairs between unnatural and native bases. The results are reported in Table 1. Relative to the most stable mispair (**PIM**:dC), the **PIM**:**PIM** self-pair has a melting temperature difference of 10.1 °C and

is, therefore, very selective against mispairing. For comparison, in the same sequence context, the thermal selectivities for the native dA:dT and dC:dG base pairs are only 3.8 °C and 6.4 °C.

In order for the unnatural base pairs to be of practical value, oligonucleotides containing multiple unnatural bases should maintain high stability and specificity. To examine this we determined the melting temperatures of duplex DNA containing multiple **PICS** self-pairs. The measured T_m values, displayed in Table 2, reveal that two **PICS** self-pairs result in additional duplex stability if they are separated by native pairs. Despite the decrease in duplex stability when two **PICS** self-pairs are adjacent, selectivity against mispairing with native bases remains reasonably high. Therefore, oligonucleotides containing at least two **PICS**, in any sequence context examined, are strongly selective for correct hybridization and should make it possible to increase the stringency and information content of hybridization or encoding experiments.

Table 2. Melting temperatures T_m of duplex DNA with multiple PICS self-pairs.

Duplex[a]	T_m [°C]
5′GCGTA***XX***CATGCG3′ 3′CGCAT***XX***GTACGC5′	51.8
5′GCGTAC***X***C***X***TGCG3′ 3′CGCATG***X***G***X***ACGC5′	56.9
5′GCGTAC***X***CA***X***GCG3′ 3′CGCATG***X***GT***X***CGC5′	63.2
5′GCGTAC***X***CATGCG3′ 3′CGCATG***X***GTACGC5′	62.6
5′GCG***X***ACTCA***X***GCG3′ 3′CGC***X***TGAGT***X***CGC5′	62.0
5′GCGTACTCA***X***GCG3′ 3′CGCATGAGT***X***CGC5′	61.2
5′GCGTA***XX***CATGCG3′ 3′CGCATG***X***GTACGC5′	49.1
5′GCGTAC***X***C***X***TGCG3′ 3′CGCATG***X***GTACGC5′	51.4
5′GCGTAC***X***CA***X***GCG3′ 3′CGCATGAGT***X***CGC5′	53.5
5′GCGTACACATGCG3′ 3′CGCATGTGTACGC5′	59.2

[a] ***X*** = **PICS**.

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