Nucleic Acids

DOI: 10.1002/anie.201301659

Enantiomeric Selection Properties of β-homoDNA: Enhanced Pairing for Heterochiral Complexes**

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The analysis of the physicochemical properties of sugar-modified nucleic acids is currently at the core of intense multidisciplinary investigations including chemistry, biology, biotechnology, and medicine.[1] On one side, synthetic polymers acting as RNA/DNA mimics have extensively been devised for applications in therapy, diagnostics, and synthetic biology.[2,3] On the other side, the construction of alternative pairing systems has been explored either to consider their use as orthogonal nucleic acid candidates[4] or with the aim to potentially yield insights into the chemical evolution criteria ultimately leading to the current genetic system.[5] In all cases, structural changes of natural (deoxy)ribose cores have been established to determine profound consequences in the pairing potential of the resulting artificial nucleic acids.[6,7] In some noteworthy examples, oligonucleotide systems endowed with six-membered sugars in the backbone have been observed[8–10] to hold the singular property (unique of its kind) of pairing with homochiral complements having opposite sense of chirality. Relevant to etiology-oriented investigations on nucleic acid structure,[5] these findings could suggest the existence of a relationship between nature of the sugar backbone and chiral-selection properties of nucleic acids, thereby providing insights to enrich our understanding of the structural prerequisites for base pairing. From a comparative analysis of the pairing behavior of six-membered nucleic acids[2,5–7] we perceived that, despite the large structural differences, oligonucleotide systems capable of iso- and heterochiral hybridization (Figure 1) shared preorganized carbohydrate conformations with equatorially-oriented nucleobases.[11] This observation took us to wonder if

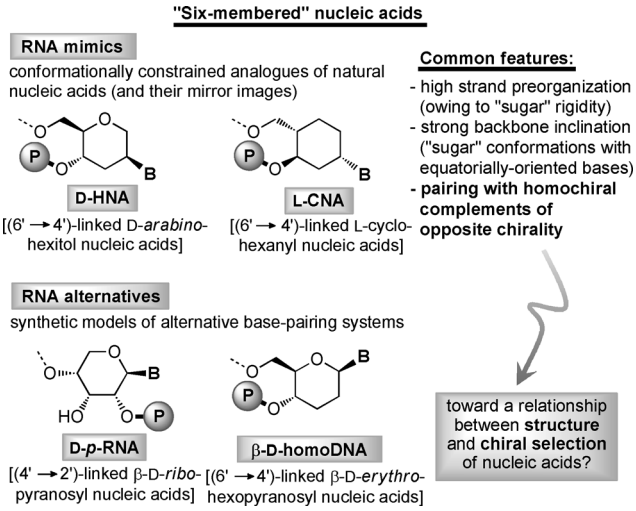

Figure 1. Sugar-modified nucleic acids displaying pairing aptitude for homochiral complements of opposite chirality.

such an arrangement of the aglycon moiety, especially whereas inducing strong backbone-base inclination[6] or even enabling formation of quasilinear oligomeric structures,[5–8] could lead sugar chirality not to be crucial in hybridization processes. In view of systematic investigations aimed at addressing this question, we herein considered the chiral selection properties of the well-known[5,12] pairing system composed of (6′→4′)-linked β-*erythro*-hexopyranosyl nucleotides (β-homoDNA; Figure 1). Based on above assumptions and early experimental data,[8] we reasoned that the strongly inclined[5,12] complexes provided by the "all-equatorial" pyranose backbone of β-homoDNA could make the latter an interesting candidate displaying potential for heterochiral hybridization.

An investigation into the enantioselectivity of the hybridization processes of β-homoDNA required access to oligomeric sequences in both enantiomeric forms (β-D- and β-L-homoDNA). From a synthetic standpoint, while access to D-hexopyranosyl nucleosides was easily obtained by a carbohydrate-based route,[13] the synthesis of the corresponding L-enantiomers under the same reaction conditions was hampered by the limited commercial availability of almost all L-hexoses. In an alternative path, our long studied de novo approach to L-monosaccharides[14] and other structurally-related compounds[9] was recently exploited[15] for the preparation of the L-nucleosides **2a**,**b** (T and A^{Bz} acting as model nucleobases) from the homologating agent **1** (Scheme 1).

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[**] Financial support from KU Leuven (GOA/10/13) and FWO (Flemish Scientific Research grant G.0784.11N) is gratefully acknowledged.
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201301659.

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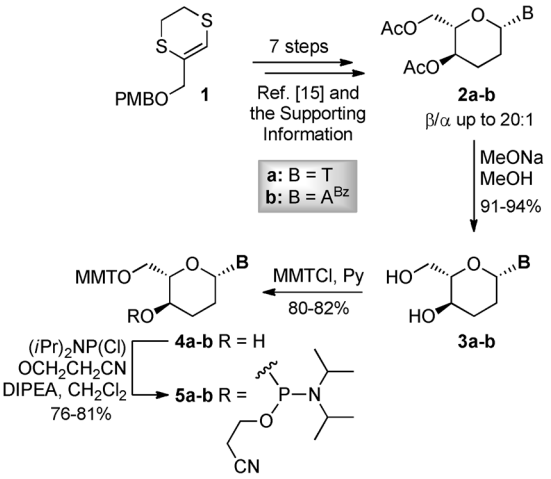

Scheme 1. The β-L-*erythro*-hexopyranosyl nucleotides **5a**,**b** as building blocks for β-L-homoDNA synthesis. DIPEA = diisopropylethylamine, MMTCl = *p*-monomethoxytrityl chloride, PMB = *para*-methoxybenzyl, Py = pyridine.

Synthesis was based on a key stereoselective *N*-glycosidation involving in situ anomerization of α/β nucleosides (β/α up to 20:1). Conversion of **2a**,**b** into the phosphoramidite nucleotides **5a**,**b** was then carried out under common reaction conditions (Scheme 1). Fully modified sequences containing β-L-*erythro*-hexopyranosyl nucleotides were synthesized using the phosphoramidite method on solid support.[13a]

In early annealing experiments we assessed that β-L-homoDNA [L-$(\mathbf{B}^h)_n$, **B** = A/T] formed isochiral self-complementary duplexes (*ds*-β-L-homoDNA) with the same melting profiles as those reported for *ds*-β-D-homoDNA[16] (Table 1, entries 1–3). Besides common L-$\mathbf{A}^h$:L-$\mathbf{T}^h$ pairing, formation of L-$\mathbf{A}^h$:L-$\mathbf{A}^h$ complexes was indicated by the UV melting curve of L-$(\mathbf{A}^h)_6$ (entry 1), and strongly suggested intermolecular self-association. Pairing priority[16] ($\mathbf{A}^h$:$\mathbf{A}^h$ > $\mathbf{A}^h$:$\mathbf{T}^h$) was demonstrated by comparing the T_m value (55 °C) of the self-complementary strand (entry 2) with the higher T_m value (58 °C) exhibited by the shorter, self-complementary sequence comprising both $\mathbf{A}^h$:$\mathbf{A}^h$ and $\mathbf{A}^h$:$\mathbf{T}^h$ base pairs.

Table 1: Thermal stability studies of complexes containing β-(D- and/or L-) homoDNA. Melting points were determined in 0.1 M NaCl, 20 mM KH_2PO_4 (pH 7.5), 0.1 mM Na_2EDTA (4 μM concentration of each strand unless otherwise specified).

Entry	Oligonucleotide	Sequence	Complement (T_m [°C])	
			D-homoDNA	L-homoDNA
1	L-homoDNA	L-$(\mathbf{A}^h)_6$	n.d.	46[a,b]
2	L-homoDNA	L-$(\mathbf{A}^h)_6(\mathbf{T}^h)_6$	n.d.	55[a]
3	L-homoDNA	L-$(\mathbf{A}^h)_6(\mathbf{T}^h)_4$	n.d.	58[a]
4	D-DNA	D-$(dT)_{13}$	–[c]	–[c]
5	D-DNA	D-$(dA)_{13}$	–[c]	–[c]
6	D-RNA	D-$(rU)_{13}$	–[c]	–[c]
7	D-RNA	D-$(rA)_{13}$	–[c]	–[c]
8	D-CNA	D-$(\mathbf{T}^c)_{13}$	>90[b]	29,[d,e] >90[b]
9	D-CNA	D-$(\mathbf{A}^c)_{13}$	22[d,f]	60
10	D-HNA	D-$(\mathbf{T}^H)_{13}$	86	78
11	D-homoDNA	D-$(\mathbf{A}^h)_{13}$	35, >90[b]	85
12	D-homoDNA	D-$(\mathbf{A}^h)_{13}$[g]	83[b]	87

[a] Used 8 μM of the self-complementary sequence. [b] Referred to an $\mathbf{A}^h$:$\mathbf{A}^h$ association. [c] No clear cooperative transition detected. [d] Determined by evaluation in the mirror-image world (Ref. [17]). [e] UV, CD, and PAGE data suggested formation of heterochiral duplexes and triplexes. [f] Ttaken from Ref. [18]. [g] Melting points determined by CD analysis. n.d. = not determined.

The pairing properties of β-L-homoDNA were then examined in annealing studies with some (un)natural D-complements (Table 1, entries 4–12). Sugar-modified oligonucleotide systems adopt quasilinear structures by virtue of the equatorial nucleobase arrangement.[5–8] The stability of the resulting complexes was compared with those containing its D-enantiomer (β-D-homoDNA). Formation of isochiral/heterochiral hybrids was either directly determined or indirectly deduced by studies in the "mirror-image world".[17]

Neither in homopurine nor homopyrimidine form did β-L-homoDNA show any significant pairing aptitude toward natural complements (Table 1, entries 4–7). Analogous results have already been reported for β-D-homoDNA.[5,18] In contrast, β-L-homoDNA exhibited from good to excellent hybridization properties when annealed with preorganized D-oligonucleotide partners (entries 8–12). Notably, the stability of the complexes obtained between strands of opposite chirality was generally higher than that of the corresponding isochiral associations. For example, β-L-homoDNA formed stronger complexes with D-CNA (T_m up to 60 °C) than those formed between β-D-homoDNA and D-CNA (T_m up to 22 °C; entries 8–9). Conversely, when annealed with D-HNA, β-L-homoDNA gave hybrids of comparable stability (entry 10). In line with previous observations,[7] the stability of heterochiral complexes increased with the preorganization of nucleic acid complements (entries 8–10). Along this line, annealing experiments between homochiral β-homoDNA complements having the same or opposite sense of chirality (β-homoDNA acting as the most preorganized pairing system) were eventually performed (entries 11 and 12). Compared with the weak transition likely related to the isochiral duplex $\mathbf{A}^h$:$\mathbf{T}^h$ (T_m = 35 °C), the melting curve observed from mixing equimolar amounts of D-$(\mathbf{A}^h)_{13}$ and L-$(\mathbf{T}^h)_{13}$ was referred to formation of a complex having far greater stability (T_m = 85 °C). Unexpectedly, when examining the UV melting behavior of the heterochiral mixture, no trace of the exceedingly stable isochiral $\mathbf{A}^h$:$\mathbf{A}^h$ association (T_m > 90 °C) was detected. Conversely, such an association largely occurred when D-$(\mathbf{A}^h)_{13}$ and D-$(\mathbf{T}^h)_{13}$ were mixed[19] (entry 11). We were also surprised to find some discrepancies between UV- and CD-melting measurements of the same mixtures (entry 12). In the latter case, the heterochiral $\mathbf{A}^h$:$\mathbf{T}^h$ association (T_m = 87 °C) resulted thermodynamically more stable than both the isochiral $\mathbf{A}^h$:$\mathbf{T}^h$ (T_m not detected)[20] and $\mathbf{A}^h$:$\mathbf{A}^h$ complexes (T_m = 83 °C).[21]

Because of their singular behavior, β-homoDNA-based complexes were subjected to further comparative studies. CD analysis of iso- and heterochiral mixtures (Figure 2) confirmed hybrid formation between β-homoDNA complements with opposite sugar chirality. Likewise, large conformational differences among these complexes were suggested, as a result of the presence of oligomeric strands providing matching/mismatching chiroptical contributions. For example, although all complexes displayed almost superimposable

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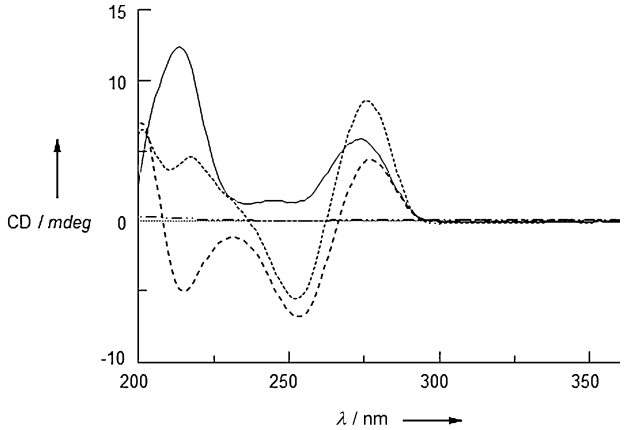

Figure 2. Normalized CD spectra of: *ds*-D-$(\mathbf{A}^h)_{13}$ (••••); D-$(\mathbf{A}^h)_{13}$ + D-$(\mathbf{T}^h)_{13}$ (1:1 mixture; -----); D-$(\mathbf{A}^h)_{13}$ + L-$(\mathbf{T}^h)_{13}$ (1:1 mixture; ——); and D-$(\mathbf{T}^h)_{13}$ (–••–). All measurements were taken at 20 °C in 0.01 M tris-HCl, 0.15 M NaCl buffer (pH 7.0).

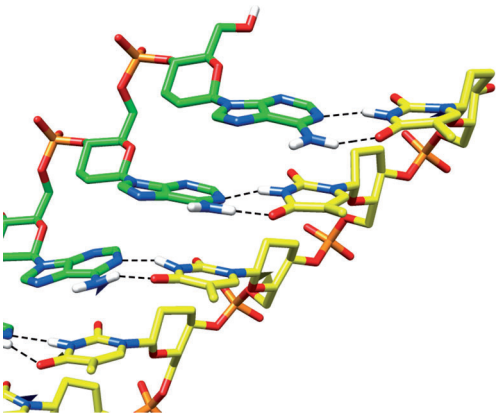

Figure 4. Close view of the simulated D-$(\mathbf{A}^h)_{13}$:L-$(\mathbf{T}^h)_{13}$ duplex. Green: carbon atoms in the D-$(\mathbf{A}^h)_{13}$ strand. Yellow: carbon atoms in the L-$(\mathbf{T}^h)_{13}$ strand.

positive bands around $\lambda = 275$ nm, only isochiral duplexes showed a negative band around $\lambda = 250$ nm. At about the same wavelength, the heterochiral complex exhibited a very weak absorption. Isochiral and heterochiral $\mathbf{A}^h$- and $\mathbf{T}^h$-containing mixtures also roughly displayed mirrored Cotton effects around $\lambda = 215$ nm (Figure 2).

A clear view of the pairing behavior of β-homoDNA was provided by PAGE analysis under nondenaturating conditions (Figure 3). While pairing of isochiral strands led to co-

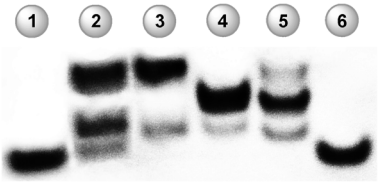

Figure 3. 20% PAGE under nondenaturing conditions of: D-$(\mathbf{T}^h)_{13}$ (lane 1); D-$(\mathbf{A}^h)_{13}$ + D-$(\mathbf{T}^h)_{13}$ (1:1 mixture; lane 2); D-$(\mathbf{A}^h)_{13}$ (lane 3); D-$(\mathbf{A}^h)_{13}$ + L-$(\mathbf{T}^h)_{13}$ (1:1 mixture; lane 4); D-$(\mathbf{A}^h)_{13}$ + L-$(\mathbf{T}^h)_{13}$ (2:1 mixture; lane 5); L-$(\mathbf{T}^h)_{13}$ (lane 6). Measurements were taken in 0.01 M Tris-HCl, 0.15 M NaCl, pH 7.0 (2 μM concentration of each oligonucleotide strand).

occurrence of $\mathbf{A}^h$:$\mathbf{T}^h$ and $\mathbf{A}^h$:$\mathbf{A}^h$ duplexes (lane 2), the latter totally disappeared owing to formation of a complex of slightly faster mobility, thus resulting from pairing of heterochiral $\mathbf{A}^h$ and $\mathbf{T}^h$ strands (lane 4). The supposed formation of triplexes such as D-$\mathbf{A}^h$:D-$\mathbf{A}^h$:L-$\mathbf{T}^h$ (which could explain disappearance of the $\mathbf{A}^h$:$\mathbf{A}^h$ duplex) was also realistically ruled out (lane 5).

The heteroduplex shape and stability were eventually explored using MD simulations (Figure 4). Although slightly right-handed, the antiparallel D-$(\mathbf{A}^h)_{13}$:L-$(\mathbf{T}^h)_{13}$ duplex model displayed almost no helicity, with an average helical twist of 6.0°, as well as a strong backbone-base inclination with a predominance for interstrand over intrastrand base stacking. Sugar substituents adopted a classic all-equatorial arrangement (corresponding to a $^4C_1$ conformation for β-D-homoDNA and a $^1C_4$ form for β-L-homoDNA). In line with experimental data, the duplex model was found to be very stable, as the temperature increase to 360 K did not affect its structural integrity.

Interestingly, striking structural differences arose from comparison of the heteroduplex with a previous[6] MD simulation of the isochiral *ds*-D-homoDNA. Contrary to the quasilinear shape of the former, the latter is known[6,12] to adopt a helical structure. In addition, the high backbone-base inclination value of the isochiral duplex model (η_B 37°) was lower than that calculated for the heteroduplex (η_B 46°). In view of the correlation between backbone-base inclination and interstrand stacking in nucleic acid duplexes,[22] it is reasonable to hypothesize a greater interstrand stacking contribution in the heterochiral complex, which could explain, in comparison with the isochiral duplex, its higher thermodynamic stability.

In summary, the preliminary analysis of the pairing properties of β-L-homoDNA has revealed that, despite sugar stereochemistry, it is able to strongly pair with homochiral D-complements, in many cases with a greater stability than that observed in the corresponding isochiral complexes. The heteroduplex composed of enantiomeric β-homoDNA complements has especially deserved attention owing to a notable thermodynamic stability, it can be currently considered as the strongest association between homochiral oligomers of opposite sense of chirality. The selectivity observed during formation (preferred to the competitive, isochiral self-complementary pairing process) also represents, to the best of our knowledge, an unprecedented event among either natural or artificial nucleic acids. From an etiological standpoint, the reversed enantioselectivity in the pairing properties of β-homoDNA underlines the "unsuitability" of hexose nucleic acids (β-homoDNA acting as a model system) as "potentially natural" RNA alternatives. Most generally, our results strengthen the hypothesis of a role played by the sugar unit of six-membered nucleic acids in the alteration of the stereoselectivity of the hybridization processes. Previous and current experimental clues highlight the importance of sugar conformation (involving equatorial

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nucleobase arrangement), although participation of other factors (above all, sugar rigidity) has also been herein suggested. In-depth studies aimed at shedding light on this topic are currently ongoing and will be published in due course.

Received: February 26, 2013
Published online: May 13, 2013

Keywords: carbohydrates · chirality · heterochiral hybridization · homoDNA · nucleic acids

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