# Enantiomeric Selection Properties of β-homo-DNA: Enhanced Pairing for Heterochiral Complexes

Daniele D'Alonzo,* Jussara Amato, Guy Schepers, Matheus Froeyen, Arthur Van Aerschot, Piet Herdewijn* and Annalisa Guaragna

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 core 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 of 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 took us to wonder if such an arrangement of the aglycon moiety, especially whether inducing strong backbone-base inclination[6] or even enabling formation of quasi-linear oligomeric structures,[5-8]

could bring 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-homo-DNA", 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-homo-DNA could make the latter an interesting candidate displaying potential for heterochiral hybridization.
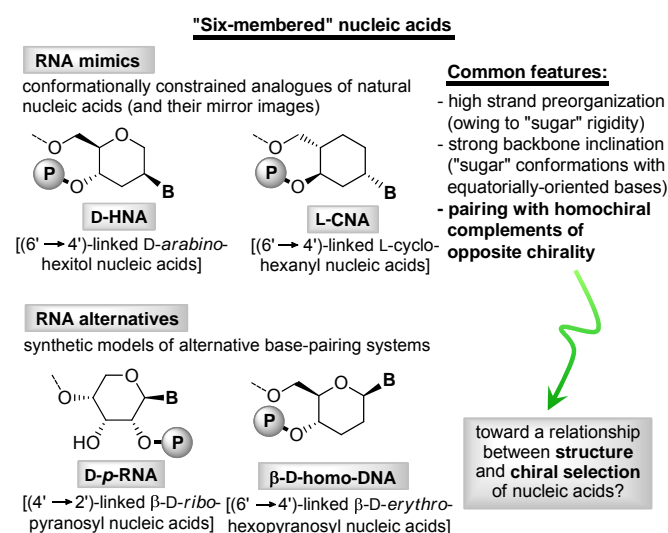
Figure 1. Sugar-modified nucleic acids displaying pairing aptitude for homochiral complements of opposite chirality.

An investigation into the enantioselectivity of the hybridization processes of β-homo-DNA required access to oligomeric sequences in both enantiomeric forms (β-D- and β-L-homo-DNA). 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 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 L-nucleosides **2a-b** (T and A[Bz] acting as model nucleobases) from homologating agent **1** (Scheme 1). 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 phosphoramidite nucleotides **5a-b** was then carried out under common conditions (Scheme 1). Fully-modified sequences containing β-L-*erythro*-hexopyranosyl nucleotides were synthesized using the phosphoramidite method on solid support.[13a]

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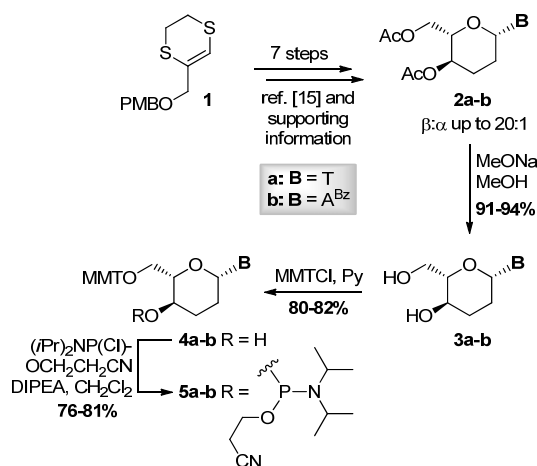
Scheme 1. β-L-*Erythro*-hexopyranosyl nucleotides **5a-b** as building blocks for β-L-homo-DNA synthesis.

In early annealing experiments we assessed that β-L-homo-DNA [L-($\mathbf{B^h}$)$_n$, **B** = A/T] formed isochiral self-complementary duplexes (*ds*-β-L-homo-DNA) with the same melting profiles as those reported for *ds*-β-D-homo-DNA[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), which strongly suggested intermolecular self-association. Pairing priority[16] ($\mathbf{A^h}$:$\mathbf{A^h}$ > $\mathbf{A^h}$:$\mathbf{T^h}$) was demonstrated comparing the T_m value (55 °C) of the self-complementary strand (entry 2) with the higher T_m (58 °C) exhibited by the shorter, self-complementary sequence comprising both $\mathbf{A^h}$:$\mathbf{A^h}$ and $\mathbf{A^h}$:$\mathbf{T^h}$ basepairs.

The pairing properties of β-L-homo-DNA were then examined in annealing studies with some (un)natural D-complements (entries 4-12). Sugar-modified oligonucleotide systems adopt quasi-linear 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-homo-DNA). Formation of isochiral/heterochiral hybrids was either directly determined or indirectly deduced by studies in the "mirror-image world".[17]

Either in homopurine or homopyrimidine form, β-L-homo-DNA did not show any significant pairing aptitude to natural complements (entries 4-7). Analogous results have already been reported for β-D-homo-DNA.[5,18] On the other hand, β-L-homo-DNA exhibited from good to excellent hybridization properties 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-homo-DNA formed stronger complexes with D-CNA (T_m up to 60°C) than those between the latter and β-D-homo-DNA (T_m up to 22°C, entries 8-9). Conversely, when annealed with D-HNA, β-L-homo-DNA gave hybrids of comparable stability (entry 10). In line with previous observations,[7] the stability of heterochiral complexes increased with preorganization of nucleic acid complements (entries 8-10). Along this line, annealing experiments between homochiral β-homo-DNA complements having same or opposite sense of chirality (β-homo-DNA acting as the most preorganized pairing system) were eventually performed (entries 11-12). Compared with the weak transition likely related to isochiral duplex $\mathbf{A^h}$:$\mathbf{T^h}$ (T_m 35°C), the melting curve observed mixing equimolar amounts of D-($\mathbf{A^h}$)$_{13}$ and L-($\mathbf{T^h}$)$_{13}$ was referred to formation of a complex of far higher 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, it was found as largely occurring 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 last case, the heterochiral $\mathbf{A^h}$:$\mathbf{T^h}$ association (T_m 87°C) even 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]

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

| Entry	Oligonucleotide (sequence)	Complement (T_m [°C])	
		D-homo-DNA	L-homo-DNA
1	L-homo-DNA, L-($\mathbf{A^h}$)$_6$	ND	46[a,b]
2	L-homo-DNA, L-($\mathbf{A^h}$)$_6$($\mathbf{T^h}$)$_6$	ND	55[a]
3	L-homo-DNA, L-($\mathbf{A^h}$)$_6$($\mathbf{T^h}$)$_4$	ND	58[a]
4	D-DNA, D-(d$\mathbf{T}$)$_{13}$	/[c]	/[c]
5	D-DNA, D-(d$\mathbf{A}$)$_{13}$	/[c]	/[c]
6	D-RNA, D-(r$\mathbf{U}$)$_{13}$	/[c]	/[c]
7	D-RNA, D-(r$\mathbf{A}$)$_{13}$	/[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-homo-DNA, D-($\mathbf{A^h}$)$_{13}$	35, >90[b]	85
12	D-homo-DNA, D-($\mathbf{A^h}$)$_{13}$[g]	83[b]	87

ND: not determined; [a] 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] taken from ref. [18]; [g] melting points determined by CD analysis.

Because of their singular behaviour, β-homo-DNA-based complexes were subjected to further comparative studies. CD analysis of iso- and heterochiral mixtures (Figure 2) confirmed hybrid formation between β-homo-DNA complements with opposite sugar chirality; likewise, large conformational differences among these complexes were suggested, as result of the presence of oligomeric strands providing matching/mismatching chiroptical contributions. For example, although all complexes displayed almost superimposable positive bands around 275 nm, only isochiral duplexes showed a negative band around 250 nm; at about the same wavelength, the heterochiral complex exhibited instead a very weak absorption. Isochiral and heterochiral $\mathbf{A^h}$ and $\mathbf{T^h}$ containing mixtures also displayed roughly mirrored Cotton effects around 215 nm (Figure 2).
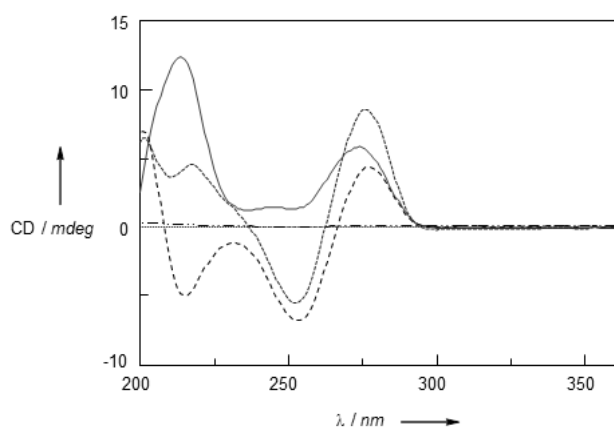
Figure 2. Normalized CD spectra of: (·····) *ds*-D-(**A**[h])$_{13}$; (– –) D-(**A**[h])$_{13}$ + D-(**T**[h])$_{13}$ (1:1 mixture); (—) D-(**A**[h])$_{13}$ + L-(**T**[h])$_{13}$ (1:1 mixture); (–·–·) D-(**T**[h])$_{13}$. All measurements were taken at 20°C in 0.01 M tris-HCl, 0.15M NaCl buffer (pH 7.0).

A clear view of the pairing behavior of β-homo-DNA was provided by PAGE analysis (Figure 3). While pairing of isochiral strands led to co-occurrence of **A**[h]:**T**[h] and **A**[h]:**A**[h] duplexes (lanes 2), the latter totally disappeared owing to formation of a complex of slightly faster mobility, resulting from pairing of heterochiral **A**[h] and **T**[h] strands (lane 4). The supposed formation of triplexes such as D-**A**[h]:D-**A**[h]:L-**T**[h] (which could explain disappearance of the **A**[h]:**A**[h] duplex) was also realistically ruled out (lane 5).
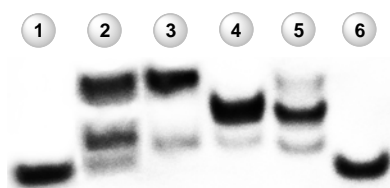
Figure 3. 20% PAGE under non-denaturing conditions of: D-(**T**[h])$_{13}$ (lane 1); D-(**A**[h])$_{13}$ + D-(**T**[h])$_{13}$ 1:1 mixture (lane 2); D-(**A**[h])$_{13}$ (lane 3); D-(**A**[h])$_{13}$ + L-(**T**[h])$_{13}$, 1:1 mixture (lane 4); D-(**A**[h])$_{13}$ + L-(**T**[h])$_{13}$, 2:1 mixture (lane 5); L-(**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).

Heteroduplex shape and stability were eventually explored using MD simulations (Figure 4). Although slightly right-handed, the antiparallel D-(**A**[h])$_{13}$:L-(**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-homo-DNA and a $^1C_4$ form for β-L-homo-DNA). In line with experimental data, the duplex model was found to be very stable, as temperature increase up to 360K 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-homo-DNA. Contrarily to the quasi-linear 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 however 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's 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.
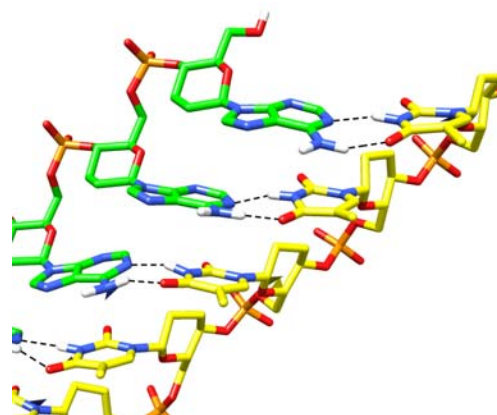
Figure 4. Close view of the simulated D-(**A**[h])$_{13}$:L-(**T**[h])$_{13}$ duplex. Green: carbon atoms in the D-(**A**[h])$_{13}$ strand; yellow: carbon atoms in the L-(**T**[h])$_{13}$ strand.

In summary, the preliminary analysis of the pairing properties of β-L-homo-DNA has revealed that, despite sugar stereochemistry, it is able to strongly pair with homochiral D-complements, in most cases with a far higher stability than that observed in the corresponding isochiral complexes. The heteroduplex composed of enantiomeric β-homo-DNA complements has especially deserved attention: owing to a notable thermodynamic stability, the latter currently stands as the strongest association between homochiral oligomers of opposite sense of chirality. The selectivity observed during hybrid 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-homo-DNA underlines the "unsuitability" of hexose nucleic acids (β-homo-DNA 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 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.

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[19]   Studies to understand this phenomenon are underway; an uncommon kinetic control ruling the pairing process has been preliminarily hypothesized to explain our experimental data.

[20]   Isochiral A^h:T^h duplex was not detected by CD-melting analysis likely due to its limited occurrence with respect to the coexisting A^h:A^h association (compare the UV-melting spectrum of the same mixture).

[21]   It must be stressed that UV- and CD-melting experiments measure different properties of a duplex, therefore different results are not contradictory. Efforts aimed at a full rationalization of this behavior are however in progress.

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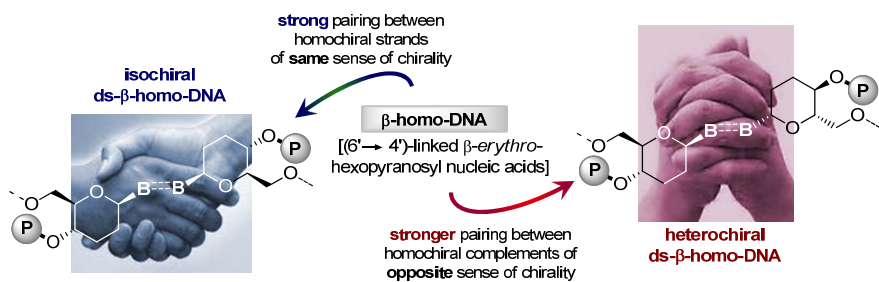
De gustibus: β-homo-DNA holds the singular property of pairing with homochiral complements of opposite chirality, with a far higher stability than that observed in the corresponding isochiral complexes. Relevant to etiological investigations on nucleic acid structure, these results suggest the existence of a relationship between carbohydrate structure and stereoselectivity of the hybridization processes of the corresponding nucleic acids.