Anomeric DNA: Functionalization of α-D Anomers of 7-Deaza-2′-deoxyadenosine and 2′-Deoxyuridine with Clickable Side Chains and Click Adducts in Homochiral and Heterochiral Double Helices

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In memory of Prof. Dr. Helmut Vorbrüggen, a passionate chemist, a dear colleague, and a close friend. He will be remembered as a leading personality for his contributions to nucleoside chemistry.

Abstract: Anomeric base pairs in heterochiral DNA with strands in the α-D and β-D configurations and homochiral DNA with both strands in α-D configuration were functionalized. The α-D anomers of 2′-deoxyuridine and 7-deaza-2′-deoxyadenosine were synthesized and functionalized with clickable octadiynyl side chains. Nucleosides were protected and converted to phosphoramidites. Solid-phase synthesis furnished 12-mer oligonucleotides, which were hybridized. Pyrene click adducts display fluorescence, a few of them with excimer emission. \( T_m \) values and thermodynamic data revealed the following order of duplex stability \( \alpha/\alpha-D \gg \beta/\beta-D \geq \alpha/\beta-D \). CD spectra disclosed that conformational changes occur during hybridization. Functionalized DNAs were modeled and energy minimized. Clickable side chains and bulky click adducts are well accommodated in the grooves of anomeric DNA. The investigation shows for the first time that anomeric DNAs can be functionalized in the same way as canonical DNA for potential applications in nucleic acid chemistry, chemical biology, and DNA material science.

Introduction

Anomeric DNA is formed when one strand of a duplex is in the α-D and the other in the β-D configuration,[1,2] When DNA is constructed from two strands in the α-D configuration, homochiral α/α-DNA is generated; the anomeric counterpart to canonical DNA. The strand orientation in heterochiral α/β-DNA is parallel, whereas homochiral α/α-DNA displays an antiparallel alignment.[3,4] Here, the terms homochiral and heterochiral correspond solely to the stereochemistry at the anomeric center and not to α/β configuration. Recently, 2-aminoadenine and the related 8-aza-7-deazaadenine nucleobases (purine numbering is used throughout the results and discussion section) were used as replacements of the adenine moiety in an adenine–thymine pair. As a result, a significant stabilization of modified duplexes over that with canonical bases was observed. Also, the capability of anomeric nucleosides to form silver-mediated base pairs was demonstrated on the basis of an anomeric α-D/β-D dC-dC metal base pair.[5]

Herein, we report on the functionalization of the 2′-deoxyadenosine-2′-deoxythymidine base pair. The 2′-deoxyadenosine moiety was replaced by the α-D anomer of 7-deaza-2′-deoxyadenosine and the 2′-deoxythymidine residue was substituted by anomeric 2′-deoxyuridine moieties. Both nucleosides were functionalized; the 7-deazapurine base at the 7-position and the pyrimidine base at the 5-position. Modifications were performed at one site of the base pair and double modifications at both sites. We anticipated that the positions of functionalization occurring in the major groove of canonical DNA will be also suitable for anomeric DNA and bulky residues might be well accommodated in anomeric double helices.

Octadiynyl residues with terminal triple bonds were introduced in the α-D anomer of 7-deaza-2′-deoxyadenosine (c′A) and 2′-deoxyuridine (dU) as they can be clicked to almost any other azide by the Huisgen-Meldal-Sharpless cycloaddition. Bulky pyrene azide was employed to form click adducts. To this end, nucleosides 2 and 9 were protected and converted to phosphoramidites and 12-mer oligonucleotides were synthesized. The synthesis of the oligonucleotides with α-D configuration made use of phosphoramidites of the α-D anomers of 7-deaza-2′-deoxyadenosine 12 and 2′-deoxyuridine 4 together with those of the four α-D nucleoside phosphoramidites with canonical bases. Then, strands were hybridized in various
combinations to form heterochiral and homochiral duplexes (Figure 1). Within the anomic strands, single incorporations of base-modified α-nucleosides were performed at the purine or the pyrimidine site and double modifications on both sites. Finally, copper(I)-catalyzed click reactions were executed and bulky pyrene substituents were introduced. In addition, the sequential order of heterochiral DNAs was altered to evaluate sequence dependencies between anomeric and canonical DNAs.

With duplexes in hand, temperature-dependent melting profiles were recorded and \( T_m \) values and thermodynamic data were calculated. CD-spectra were measured to detect global helical changes. Fluorescence studies with DNA pyrene adducts gave information on DNA dye conjugates. Finally, AMBER force field energy minimization were undertaken on the functionalized DNA. These studies visualize the size of bulky substituents and the available space of the grooves.

### Results and Discussion

**Synthesis and characterization of α-β anomers of 5-octadiynyl-2′-deoxyuridine (2) and 7-octadiynyl-7-deaza-2′-deoxyadenosine (9), conversion to phosphorimidites and pyrene click adducts**

The α-β iodonucleoside 1\(^{(1)}\) was chosen as starting material for the synthesis of the 5-octadiynyl-α-β 2′-deoxyuridine (2; Scheme 1). The octadiynyl side chain was introduced by Sonogashira cross-coupling (Pd\(^0\)/CuI) employing an excess of octa-1,7-diyne to assure mono-functionalization. By this, the 5-octadiynylated α-β pyrimidine nucleoside 2 was isolated in 56% yield after chromatographical purification. Protection of the 5′-OH using DMT-Cl afforded the 5′-protected nucleoside 3 (87% yield). Treatment of 3 with 2-cyanoethyl disopropylphosphoramidochloridite gave phosphoramidite 4 in 68% yield. For the synthesis of 7-octadiynylated α-β-7-deaza-2′-deoxyadenosine (9) a similar route was employed. Nucleobase anion glycosylation of 6-chloro-7-ido-7-deazapurine (5) with Hoffmann halogenase 6\(^{(5)}\) furnished the monomeric iodo nucleoside 7 and its β-α anomer. Usually, anion glycosylation is stereoselective for the β-α anomer but in this particular case (6-chloro-7-ido base 5) the α-β anomer 7 is formed as a side product in 10% yield together with the β-α anomer in 65%\(^{(6)}\). The anemic mixture can be easily separated by flash column chromatography to give the pure anomers. For the β-α anomer 14 the synthetic route to access the phosphoramidite 16 has been described,\(^{(7)}\) whereas the phosphoramidite of the α-β anomer 9 is unknown. Consequently, 7-iodinated α-β-7-deaza-2′-deoxyadenosine (8) which was obtained from 7 according to a literature protocol\(^{(8)}\) was used in the Sonogashira cross-coupling together with octa-1,7-diyne and Pd\(^0\)/CuI as catalysts. By this, the 7-octadiynyl-α-β-7-deaza-2′-deoxyadenosine 9 was obtained in 75%. Then, octadiynylated 9 was protected at the amino group with an isobutyryl residue under conditions of transient protection giving the isobutyrylated compound 10 in 60% yield. Trytlylation with DMT-Cl afforded the DMT derivative 11 (49%) and phosphorylation at 3′-OH gave the phosphorimidite 12 in 72% yield. The syntheses of the corresponding β-α nucleosides 13\(^{(9)}\) and 14\(^{(10)}\) and their phosphoramidites 15\(^{(5)}\) and 16\(^{(11)}\) have been described earlier by our laboratory.

Next, the pyrene functionalized α-β and β-α nucleosides 17 and 18 were synthesized by using the Huisgen-Meldal-Sharpless click reaction (Scheme 2)\(^{(12,13)}\). For this, the 5-octadiynylated compounds 2 and 13 were treated with an excess of pyrene azide in the presence of Cu\(^2+\) sulfate pentahydrate and ascorbic acid as reducing agent in THF/tBuOH/H\(_2\)O at RT overnight. By this, the functionalized nucleosides 17 and 18 were obtained in 62% (α-β) and 70% (β-α) yield. All new synthesized compounds were characterized by \(^1\)H, \(^13\)C NMR spectra as well as ESI-TOF mass spectra. The \(^1\)H,\(^13\)C correlated (HMBC and HSQC) NMR spectra were used to assign the \(^13\)C
NMR signals (Tables S1 and S2 in the Supporting Information). Then, fluorescence spectra were recorded in various solvents to determine solvent dependent changes (Figure S7A and B). Both anomeric dU click conjugates show almost identical spectra and the same solvent dependence. Fluorescence was high in DMSO but was low in water. Monomer emission occurred in all cases and the compounds did not show excimer emission.

**Scheme 1.** Top: Synthesis of the phosphoramidites 4 and 12 derived from 5-octadiynyl-α-d-2'-deoxyuridine (2) and 7-octadiynyl-α-d-7-deaza-2'-deoxyadenosine (9). i) CuI, Pd(PPh₃)₄, triethylamine, octa-1,7-diyne; ii) DMT–Cl, pyridine, RT, 3 h; iii) NC(OCH₂)₂P(Cl)N(iPr)₂, (iPr)₂NEt, RT; iv) KOH, TDA-1, CH₃CN, RT; v) 28% aq. NH₃/dioxane (2:1) 130 °C, 16 h; vi) 1: TMSiCl, pyridine, 30 min; 2: iBu₂O, 3 h, RT; 3: 14% aq. NH₃·H₂O, 30 min, RT. Bottom: Corresponding β-d nucleosides and phosphoramidites reported earlier.[7,9]

**Duplex stability, hypochromicity and thermodynamic data of click functionalized DNA with strands in α-d/β-d and β-d/β-d configuration**

In the first part of this section, the thermal stabilities of functionalized heterochiral 12-mer duplexes are explored and compared to their homochiral counterparts. To this end, dA or dT residues in a single dA–dT base pair were replaced by the...
Six modified building blocks were required for the synthesis of the 12-mer oligonucleotide strands all in access to the same number of deoxyguanosine derivatives and deoxyuridines.

Synthesized oligonucleotides and their molecular masses determined by MALDI-TOF mass spectrometry.

Table 1. Synthesized oligonucleotides and their molecular masses determined by MALDI-TOF mass spectrometry.

| Oligonucleotide | M.W. (calcld\(^{[a]}\)/exp.\(^{[b]}\)) | Oligonucleotide | M.W. (calcld\(^{[a]}\)/exp.\(^{[b]}\)) |
|-----------------|---------------------------------|-----------------|---------------------------------|
| ODN-1 \(\alpha\)-5′-d(TCA TAA CGT GAT) | 3644.4/3644.0 | ODN-12 \(\beta\)-5′-d(TCA TAA CGT GAT) | 3644.4/3646.4 |
| ODN-2 \(\alpha\)-5′-d(TCA TAA CG2 GAT) | 3734.5/3734.5 | ODN-13 \(\beta\)-5′-d(ATC CAG TTA TGA) | 3644.4/3643.4 |
| ODN-3 \(\alpha\)-5′-d(TCA TAA C17 G GAT) | 3991.8/3990.5 | ODN-14 \(\beta\)-5′-d(ATG TAT GAC CTA) | 3747.6/3747.2 |
| ODN-4 \(\alpha\)-5′-d(TAG GTC AAT ACT) | 3644.4/3644.4 | ODN-15 \(\beta\)-5′-d(TAG GTC AAT ACT) | 3747.6/3745.7 |
| ODN-5 \(\alpha\)-5′-d(ATC CAG TTA TGA) | 3644.4/3643.5 | ODN-16 \(\beta\)-5′-d(ATG G20 C TGA) | 4004.9/4003.7 |
| ODN-6 \(\alpha\)-5′-d(ATG ATT GCA CTA) | 3644.4/3644.1 | ODN-17 \(\beta\)-5′-d(ATG ATT G14 C CTA) | 3991.8/3990.7 |
| ODN-7 \(\alpha\)-5′-d(AGT CCG TTA TGA) | 3747.6/3747.1 | ODN-18 \(\beta\)-5′-d(CCG GAA TCC GGG) | 3646.4/3646.1 |
| ODN-8 \(\alpha\)-5′-d(AGT C19 G TTA TGA) | 4004.9/4005.7 | ODN-19 \(\beta\)-5′-d(TCA TAA C13 G TGA) | 3734.5/3733.4 |
| ODN-9 \(\alpha\)-5′-d(CCC GAA TTC TCG) | 3646.4/3647.5 | ODN-20 \(\beta\)-5′-d(ATC C14 G TTA TGA) | 3747.6/3747.2 |
| ODN-10 \(\beta\)-5′-d(TAG ATT ACT AAT) | 3643.6/3643.7 | ODN-21 \(\beta\)-5′-d(TCA TAA C18 G TGA) | 3992.8/3992.0 |
| ODN-11 \(\beta\)-5′-d(ATG ATT GCA CTA) | 3643.6/3643.7 | ODN-22 \(\beta\)-5′-d(ATC C20 G TTA TGA) | 4004.9/4005.3 |

[a] Calculated on the basis of the molecular mass of \([M+H]^+\). [b] Determined by MALDI-TOF mass-spectrometry as \([M+H]^+\) in the linear positive mode.
Table 2. T_m values and thermodynamic data for antiparallel- and parallel-strand duplexes containing α-5-octadiynyl-dU 2, β-5-octadiynyl-dU 13 and β-7-octadiynyl-c' α 14 and pyrene click conjugates15

| Heterochiral (α/β) duplexes | T_m [°C] | ΔH [kcal mol⁻¹] | ΔS [cal mol⁻¹ K⁻¹] | ΔG° [kcal mol⁻¹] |
|----------------------------|---------|-----------------|--------------------|-----------------|
| Parallel strands            |         |                 |                    |                 |
| α-5-d(TCA TAA CT G GAT) (ODN-1) | 41/21   | -66             | -183              | -9.5            |
| β-5-d(TCA TAA CT G GAT) (ODN-1) | 40/19   | -65             | -180              | -9.0            |
| α-5-d(AGT GA C CTA) (ODN-11) | 42/17   | -52            | -151             | -9.8            |
| β-5-d(AGT GA C CTA) (ODN-11) |         |                 |                    |                 |
| α-5-d(TCA TAA CT G GAT) (ODN-2) | 38/19   | -56             | -153              | -8.6            |
| β-5-d(AGT GA C CTA) (ODN-2)   | 45/17   | -59            | -157             | -10.5           |
| Double functionalization     |         |                 |                    |                 |
| α-5-d(TCA TAA CT G GAT) (ODN-1) | 37/19   | -59             | -163              | -8.4            |
| β-5-d(AGT GA C CTA) (ODN-11) | 41/15   | -52            | -140             | -8.2            |
| α-5-d(TCA TAA CT G GAT) (ODN-2) | 45/16   | -50            | -148             | -10.4           |
| β-5-d(AGT GA C CTA) (ODN-2)   | 42/17   | -49            | -123             | -10.5           |

| Homochiral (β/β) duplexes     | T_m [°C] | ΔH [kcal mol⁻¹] | ΔS [cal mol⁻¹ K⁻¹] | ΔG° [kcal mol⁻¹] |
|-------------------------------|---------|-----------------|--------------------|-----------------|
| Antiparallel strands           |         |                 |                    |                 |
| β-5-d(TCA TAA CT G GAT) (ODN-12) | 45/20   | -80             | -223              | -10.3           |
| β-3-d(TCA TAA CT G GAT) (ODN-13) | 46/19   | -81             | -227              | -10.8           |
| β-5-d(TCA TAA CT G GAT) (ODN-19) |         |                 |                    |                 |
| β-3-d(TCA TAA CT G GAT) (ODN-21) | 48/14   | -77             | -209              | -11.6           |
| β-3-d(TCA TAA CT G GAT) (ODN-22) |         |                 |                    |                 |
| Double functionalization       |         |                 |                    |                 |
| β-5-d(TCA TAA CT G GAT) (ODN-12) | 43/18   | -74             | -208              | -9.8            |
| β-3-d(AGT GA C CTA) (ODN-20)   | 47/14   | -67            | -182             | -11.0           |

[a] Measured at 260 nm at a concentration of 5 μM + 5 μM single strand at a heating rate of 1.0 °C min⁻¹ in 100 mM NaCl, 10 mM MgCl₂ and 10 mM Na-cacodylate (pH 7.0). [b] T_m values were calculated from the heating curves using the program Melting.15 [c] H = hypochromicity. [d] For duplexes containing pyrene click adducts, a concentration of 2 μM + 2 μM single strand was used.
homochiral duplexes and values between 15 to 20% were measured in both series.

Next, thermodynamic data were determined. To this end, the program Meltwin 3.0 \[12\] was used and data were extracted from melting curve shape analyses. ΔH° values were higher for homochiral DNA duplexes with both strands in β-configuration with respect to heterochiral α/β DNA. Data indicate that loss of duplex stability might be due to altered stacking forces and/or weaker H-bonding. \[13\]

The global helix conformation of duplex DNA can be monitored by CD spectra.\[14,15\] Various factors contribute to the shape of the CD-spectra: i) the conformation of the monomers which can be syn or anti, ii) the conformation of the sugar residues (N vs. S), iii) the hydrogen-bonding network formed between nucleobases, iv) stacking interactions between nucleobases or base pairs and v) the helicity of the duplex (+ or −). Previous CD experiments have shown that CD spectra of α-anomeric single strands display spectra with mirror-like Cotton effects with respect to the β-strand.\[12,23\] Complete mirror images are not expected as diastereoisomers are compared and not enantiomeric molecules with strands in α- and β-configuration. The phenomenon has been already discussed for nonfunctionalized α-D hexamer oligonucleotide duplexes with all-purine or all-pyrimidine bases in either the α- or the β-configuration.\[18\] Our DNA fragment represent a full helix turn that contains all four DNA bases in random composition with and without clickable side chains and click adducts.

According to Figure 3 remarkable strong negative Cotton effects are observed for the α-strands. These negative CD signals around 280 nm disappear when the α-strand is hybridized with the β-strand to form a duplex. Now, the Cotton effect becomes positive and displays a similar shape as the β-strand. These strong changes of the CD spectra are typical for all heterochiral DNA duplexes used in this study – modified or not. It shows that the strands adopt the conformation of the β-strand and a strong conformational change occurs during hybridization. It has been already known for canonical DNA that single strands are more flexible than duplexes. Single strands show a persistence length of a few nanometers whereas double stranded helices form stiff rods with a persistence length of around 40 nm. Here, the β-strand dictates the conformation of the final duplex. Duplex DNA is then much more difficult to bend as single stranded DNA. This was shown for canonical DNA but is now also anticipated for anomeric DNA.

Also, experimentally determined CD spectra of duplex DNAs were compared with calculated spectra (sum of the CD spectra of the single strands, Figure 3). The shape of CD curves of homochiral β/β duplexes from calculated and measured spectra is similar, whereas completely different spectra were obtained for the measured heterochiral α/β duplexes with respect to the calculated spectra. Measured α/β- duplexes exhibit a similar shape as homochiral β/β- duplexes, calculated spectra of α/β- duplexes display curves similar to α-D single strands. Apparently, upon duplex formation the β-anomer seems to dictate the sign of CD spectra and therefore the structure of the heterochiral duplexes. This phenomenon is observed for all α/β- duplexes with and without side chains. All data indicate that base pair overlaps and stacking interactions are similar in

**Figure 2.** Melting curves of heterochiral parallel α/β-0 duplexes and the corresponding homochiral antiparallel β/β-0 ones measured at 260 nm at a concentration of 5 μM + 5 μM single strand at a heating rate of 1.0 °C min⁻¹ in 100 mM NaCl, 10 mM MgCl₂, and 10 mM Na-cacodylate (pH 7.0). For duplexes containing pyrene click adducts, a concentration of 2 μM + 2 μM single strand was used.
Table 3. $T_m$ values and thermodynamic data for antiparallel- and parallel-strand duplexes containing α-7-octadiynyl-β-$c'$A, 9, β-5-octadiynyl-β-dU 13, β-7-octadiynyl-β-$c'$A 14 and pyrene click conjugates.[a]

| Heterochiral (α/β) duplexes | $T_m$ [°C] | $\Delta H$ [kcal mol$^{-1}$] | $\Delta S$ [cal mol$^{-1}$ K$^{-1}$] | $\Delta G_{m,0}$ [kcal mol$^{-1}$] | Homochiral (β/β) duplexes | $T_m$ [°C] | $\Delta H$ [kcal mol$^{-1}$] | $\Delta S$ [cal mol$^{-1}$ K$^{-1}$] | $\Delta G_{m,0}$ [kcal mol$^{-1}$] |
|----------------------------|------------|-----------------|-----------------|-----------------|----------------------------|------------|-----------------|-----------------|-----------------|
| Parallel strands           |            |                 |                 |                 | Antiparallel strands       |            |                 |                 |                 |
| β-5-d(TAG GTC AAT ACT) (ODN-10) | 45/19%    | -69             | -191            | -10.1           | β-5-d(TAG GTC AAT ACT) (ODN-10) | 47/19     | -81             | -225            | -10.9           |
| α-5-d(ATC CAG TTA G) (ODN-5)    | 44/18%    | -69             | -189            | -9.9            | β-3-d(ATT CAG TTA G) (ODN-11) | 46/18     | -82             | -231            | -10.7           |
| α-5-d(ATT CAG TTA G) (ODN-5)    | 47/15%    | -66$^{\beta d}$ | -176$^{\beta d}$ | -11.0$^{\beta d}$ | β-5-d(TAG G1C AAT ACT) (ODN-14) | 51/16$^{\delta d}$ | -76$^{\delta d}$ | -207$^{\delta d}$ | -12.2$^{\delta d}$ |
| α-5-d(ATT CAG TTA G) (ODN-5)    | 47/15%    | -66$^{\beta d}$ | -176$^{\beta d}$ | -11.0$^{\beta d}$ | β-5-d(TAG G1C AAT ACT) (ODN-14) | 51/16$^{\delta d}$ | -76$^{\delta d}$ | -207$^{\delta d}$ | -12.2$^{\delta d}$ |
| α-5-d(ATT CAG TTA G) (ODN-5)    | 47/15%    | -66$^{\beta d}$ | -176$^{\beta d}$ | -11.0$^{\beta d}$ | β-5-d(TAG G1C AAT ACT) (ODN-14) | 51/16$^{\delta d}$ | -76$^{\delta d}$ | -207$^{\delta d}$ | -12.2$^{\delta d}$ |
| Double functionalization      |            |                 |                 |                 | Double functionalization    |            |                 |                 |                 |
| β-5-d(TAG GTC AAT ACT) (ODN-10) | 43/18%    | -70             | -194            | -9.7            | β-5-d(TAG GTC AAT ACT) (ODN-10) | 45/17     | -80             | -223            | -10.4           |
| α-5-d(ATT CAG TTA G) (ODN-7)    | 46/17%    | -70$^{\beta d}$ | -190$^{\beta d}$ | -10.8$^{\beta d}$ | β-3-d(ATT CAG TTA G) (ODN-15) | 51/16$^{\delta d}$ | -71$^{\delta d}$ | -192$^{\delta d}$ | -12.0$^{\delta d}$ |
| α-5-d(ATT CAG TTA G) (ODN-8)    | 46/17%    | -70$^{\beta d}$ | -190$^{\beta d}$ | -10.8$^{\beta d}$ | β-3-d(ATT CAG TTA G) (ODN-15) | 51/16$^{\delta d}$ | -71$^{\delta d}$ | -192$^{\delta d}$ | -12.0$^{\delta d}$ |
| α-5-d(ATT CAG TTA G) (ODN-17)   | 52/18%    | -66$^{\beta d}$ | -173$^{\beta d}$ | -12.0$^{\beta d}$ | β-3-d(ATT CAG TTA G) (ODN-17) | 61/16$^{\delta d}$ | -60$^{\delta d}$ | -151$^{\delta d}$ | -13.2$^{\delta d}$ |

[a] Measured at 260 nm at a concentration of 5 μM + 5 μM single strand at a heating rate of 1.0 °C min$^{-1}$ in 100 mM NaCl, 10 mM MgCl$_2$, and 10 mM Na-cacodylate (pH 7.0). [b] $T_m$ values were calculated from the heating curves using the program Meltwin 3.0. [c] H = hypochromicity. [d] For duplexes containing pyrene click adducts, a concentration of 2 μM + 2 μM single strand was used.
heterochiral ($\alpha/\beta$) DNA with respect to homochiral ($\beta/\beta$) DNA. From the spectra of duplexes one can conclude that clickable side chains of moderate size (octadynyl) and more space demanding residues (pyrene) are well accommodated in the grooves of heterochiral $\alpha/\beta$ DNA. Thus, data confirm that the position-5 for pyrimidines and position-7 for 7-deazapurines are ideally suited for functionalization.

Figure 3. CD spectra of single- and double-stranded oligonucleotides with $\alpha$-d and $\beta$-d configurations. All measurements were performed at a concentration of 5 $\mu$M + 5 $\mu$M single strand in 100 mM NaCl, 10 mM MgCl$_2$, and 10 mM Na-cacodylate, pH 7.0. The cell path length of the cuvette for the CD measurements was 5 mm. For pyrene click adducts, a 2 $\mu$M + 2 $\mu$M single strand concentration was used. Black and red curves show the CD spectra of the single strands. Blue curves show the calculated spectra (sum of the CD spectra of the single strands). Green curves show the experimentally determined spectra.
Functionalized homochiral DNA with both side chains in α-β configuration

Next, duplexes were studied with both strands in an α-β configuration. To determine the strand stoichiometry, so-called mixing experiments of a series of homochiral duplexes were performed. For each synthetic duplex, a series of mixtures were prepared with varying ratios of oligonucleotide and a constant total oligonucleotide concentration. The absorbance of each mixture was measured three times at a wavelength of 260 nm resulting in a titration graph (Figures 4 and S2). According to Figure 4, all duplexes showed one to one strand stoichiometry confirming that only duplexes are formed and that the formation of other assemblies, for example triplexes, is excluded.

Figure 5 shows melting curves for α/α-β duplexes measured at 260 nm. According to this figure, all curves showed cooperative melting with high $T_m$ values around 60 °C. From that thermodynamic data were calculated (Table 4). Single modification of a dT residue by the side-chain derivative 2 shows a significantly higher $T_m$ value than replacement of dA by the derivative 9. On the contrary, the α-anomeric dU click adduct 17 retains the $T_m$ value of the nonfunctionalized duplex ODN-1-ODN-5. Apparently, side-chain derivatives with α-dU modification have no negative impact on the stability of homochiral α/α DNA.

Compared to that 7-deazapurine functionalized duplexes are generally less stable. This phenomenon is also apparent from duplexes with double modifications and is in line with previous results on homochiral DNA formed by both strands in β-β configuration. Hypochromicities were low for α/α duplexes (ca. 12%) with respect to α/β or β/β duplexes (ca. 20%). Obviously, the base overlap is significantly different in homochiral α/α DNA compared to β/β or α/β DNA. Nevertheless, base pairing is extremely strong.

From the thermodynamic data it is obvious that a favorable enthalpy is responsible for this phenomenon. Encouraged by these results, we synthesized the α-β anomic counterpart of the self-complementary Dickerson Drew dodecamer β-5′-d(CGCGAATTCGCG)2 (ODN-18), namely α-5′-d(CGCGAATTCGCG)2 (ODN-9). The $T_m$ of this α-anomeric Dickerson duplex was extremely high under buffer conditions used in this manuscript and a complete melting profile could not be recorded. Consequently, a low salt buffer was chosen. Now, a complete sigmoidal melting curve was observed with a $T_m$ value of 75 °C.
Table 4. $T_m$ values and thermodynamic data for antiparallel- and parallel-strand duplexes containing α-7-octadiynyl-c7A9, α-5-octadiynyl-dU2 and pyrene click conjugates.16

| Homochiral (α/α) duplexes with antiparallel strands and single modifications | $T_m [^\circ C]$ | $\Delta H$ [kcal mol$^{-1}$] | $\Delta S$ [cal mol$^{-1}$ K$^{-1}$] | $\Delta G_{25} [\text{kcal mol}^{-1}]$ |
|---|---|---|---|---|
| α-5’-dT(CTA AAT CTT GAT) (ODN-1) | 62/14 | -118 | -325 | -17.0 |
| α-3’-d(AGT ATT GAC CTA) (ODN-5) | 59/13 | -94 | -255 | -14.7 |
| α-5’-dT(CTA AAT CTT GAT) (ODN-1) | 57/14 | -73 | -191 | -13.4 |
| α-3’-d(AGT ATT GAC CTA) (ODN-7) | 62/14 | -119 | -327 | -17.1 |
| α-5’-dT(CTA AAT CTT GAT) (ODN-1) | 61/12 | -109 | -298 | -16.2 |

Homochiral (α/α) duplexes with double modification

| Homochiral (α/α) duplexes with double modification | $T_m [^\circ C]$ | $\Delta H$ [kcal mol$^{-1}$] | $\Delta S$ [cal mol$^{-1}$ K$^{-1}$] | $\Delta G_{25} [\text{kcal mol}^{-1}]$ |
|---|---|---|---|---|
| α-5’-d(CTA AAT CTT GAT) (ODN-2) | 58/12 | -81 | -218 | -13.6 |
| α-3’-d(AGT ATT GAC CTA) (ODN-7) | 58/11 | -80 | -213 | -14.1 |
| α-5’-d(CTA AAT CTT GAT) (ODN-2) | 58/11 | -75 | -197 | -13.7 |
| α-3’-d(AGT ATT GAC CTA) (ODN-7) | 57/12 | -60 | -153 | -12.5 |

[a] Measured at 260 nm at a concentration of 5 μM - 5 μM single strand at a heating rate of 1.0 °C min$^{-1}$ in 100 mM NaCl, 10 mM MgCl2, and 10 mM Na cacodylate (pH 7.0). [b] $T_m$ values were calculated from the heating curves using the program Meltwin 3.0.[12] [c] H% corresponds to hypochromicity in %. [d] For duplexes containing pyrene click adducts, a concentration of 2 μM + 2 μM single strand was used. A few single stranded α-β oligonucleotides showed weak cooperative melting which disappeared after hybridization (data are shown in Table S3 and Figure S4).

(Figure S4). The β-configurated Dickerson dodecamer showed a $T_m$ value of 40 °C for duplex melting. It is obvious that non-self-complementary and also self-complementary α/α duplexes exhibit a much higher stability than those with β/β-configuration.

To get information on global changes of oligonucleotide duplexes formed by two α-β strands CD spectra were measured. In Figure 6A, B, the measured and calculated spectra of oligonucleotide duplexes with α/α-configuration as well as spectra of the corresponding single strands are displayed. All CD spectra showed maxima with negative Cotton effects around 278 nm. This is different to the CD spectra of the heterochiral α/β duplexes all showing positive lobes. Temperature dependent CD spectra were used to determine $T_m$ values (Figure 6C, D). The $T_m$ data obtained by CD spectra (Figure 6E, F) were almost identical to those measured by UV showing high $T_m$ values around 60 °C.

A few homochiral and heterochiral duplexes reported in this study contain pyrene residues linked to the α-β or the β-β oligonucleotide strands. As discussed above, pyrene residues contribute stability to duplexes. Pyrene is a fluorescent molecule with five emission peaks (375–405 nm) and an additional band (excimer) when two pyrenes are in proximal position. As excimer emission is sensitive to environmental changes, it can be used to determine intermolecular interactions. Thus, differences might exist among the various duplexes with oligonucleotides in anomeric configuration. To this end, three duplexes were chosen with homochiral α/α, β/β strands and heterochiral α/β configuration and fluorescence measurements were performed. According to Figure 7, all duplexes show monomer fluorescence but only the homochiral duplexes ODN-3 - ODN-8 (α/α) and ODN-21 - ODN-22 (β/β) show significant excimer emission.

Molecular models of anomeric DNA and impact of nucleobases and side chains

Molecular models of anomeric DNA were constructed by Amber force field incorporated in the software package HyperChem 8.0 for Windows (Hypercube, Inc.). Original Amber parameters were used (see the Experimental Section) and no water or counter...
ions were added. All duplexes were energy minimized but not refined. To this end, the β-nucleosides from one strand of the standard β/β' duplex ODN-12-ODN-13 were replaced by α-nucleosides including those with functionalized side chains. For homochiral duplexes with both strands in α/α' configuration and antiparallel strand alignments, all β-nucleoside residues were substituted by α-nucleosides including the nucleosides with clickable side chains or pyrene click adducts. Figure 8A–C shows the impact of octadiynyl side chains and pyrene click adducts on the particular double-helix structures. Structures of the corresponding β/β' duplexes and space filling models of all structures are shown in Figures S8A–C and S9A–I. CD melting curves of duplexes obtained from temperature-dependent CD spectra of E) ODN-1-ODN-5 and F) ODN-2-ODN-5. All measurements were performed in 100 mM NaCl, 10 mM MgCl₂, and 10 mM Na-cacodylate, pH 7.0. The cell path length of the cuvette for the CD spectra was 5 mm.

**Figure 6.** A) and B) CD spectra of single- and double-stranded oligonucleotides with α-configuration. Blue curves show the calculated spectra (sum of the CD spectra of the single strands). Green curves show the experimental determined spectra. Temperature-dependent CD-spectra of C) ODN-1-ODN-5 and D) ODN-2-ODN-5. CD melting curves of duplexes obtained from temperature-dependent CD spectra of E) ODN-1-ODN-5 and F) ODN-2-ODN-5. All measurements were performed in 100 mM NaCl, 10 mM MgCl₂, and 10 mM Na-cacodylate, pH 7.0. The cell path length of the cuvette for the CD spectra was 5 mm.
and reversed Watson-Crick base pairs (Donohue pair; iG-7-deazapurines. The purine bases of the oligonucleotide duplexes containing pyrene click conjugates. All spectra were measured in 100 mM NaCl, 10 mM MgCl₂ (pH 7.0) with a concentration of 2 µM + 2 µM. The excitation wavelength was 344 nm in all cases.

Figure 7. Fluorescence emission spectra of the heterochiral α/β duplex ODN-3/ODN-16, the homochiral β/β (ODN-21 -ODN-22) and α/α (ODN-3 -ODN-8) oligonucleotide duplexes containing pyrene click conjugates. All spectra were measured in 100 mM NaCl, 10 mM MgCl₂ and 10 mM Na-cacodylate (pH 7.0) with a concentration of 2 µM + 2 µM. The excitation wavelength was 344 nm in all cases.

it is obvious, that the clickable octadiynyl residues as well as the bulky pyrene click adducts are well accommodated in heterochiral and homochiral double helices and do not disturb the global double-helix structure.

Anomeric DNA with strands in α-β and β-β configurations form duplexes with parallel strand orientation. The same strand alignment was reported for duplexes containing iG₂-iC, iG₂-iC and reversed Watson-Crick base pairs (Donohue pair; iG₂ = 2'-deoxysugarginosine, iC₂ = 2'-deoxy-5-methylisocytidine).

According to Figure 8, heterochiral duplexes with parallel strand alignment form adenine-thymine and guanine-cytosine base pairs in the Watson-Crick mode. Furthermore, Hoogsteen pairing involving nitrogen-7 of purine bases can be excluded due to the absence of this purine nitrogen. This principle has been already reported for heterochiral α/β DNA in which all purine bases of the β-strand are replaced by nonfunctionalized 7-deazapurines. The absence of purine nitrogen-7 and the replacement of the electronegative nitrogen atom of dA by an electropositive CH group (→C’A) affect the electrostatic potential in the major groove. Base stacking interactions are reduced and as a result a slight enthalpy destabilization of the c’A-dT base pair is observed with respect to the da-dT pair. Nevertheless, base pairing geometry of the Watson-Crick mode was retained.

Our Tₘ measurements and thermodynamic data indicate that this is also valid for anomeric DNA.

Conclusion

DNA possesses an intrinsic polymorphism that depends on the sequence, the structure of nucleobases, and the sugar-phosphodiester backbone. Base recognition and helix conformation play vital roles. This work reports the impact of clickable side chains and click adducts on heterochiral DNA with complementary strands in the α-α and β-β configurations and their homochiral counterparts with both strands in α-β and β-α configuration. Strand communication occurs in anomeric DNA. To this end, the α-anomer of 2'-deoxyuridine was functionalized with clickable octadiynyl side chains at the nucleobase 5-position, and the α-anomer of 7-deaza-2'-deoxyadenosine at position-7. Functionalized nucleosides were protected and converted to phosphoramidite building blocks, and oligonucleotides were synthesized. They were clicked to a bulky fluorescent pyrene azide. Heterochiral and homochiral duplexes were formed after hybridization. According to the Tₘ values and thermodynamic data, alkyl side chains of moderate size and with bulky pyrene residues are well accommodated in homochiral and heterochiral DNAs. Side-chain functionalization has only a minor effect on the stability of α/β-DNA or on DNA with both strands in the α/α configuration. Remarkably, α/α-DNAs are much more stable than their α/β and β/β counterparts. The order of duplex stability was α/α > β/β > α/β > β/α-DNAs. CD spectra of all α-β single strands show mirror-like behavior with respect to β-β oligomers. After hybridization, α/β duplexes exhibit positive Cotton effects similar to those of their β/β counterparts, whereas α/α-DNA duplexes display negative signs. The global changes in the α-strands can be attributed to conformational helix adaption during base-pair formation. In all of our cases, the β-strands dictated the sign of the CD spectrum in the final duplex. The functionalized DNAs were modeled and energy minimized. HyperChem 8.0 dynamic simulations followed by AMBER force-field energy minimization showed that heterochiral and homochiral duplexes containing clickable side chains or click adducts form stable duplex structures. Side chains have sufficient space in double helices. With the knowledge from this investigation, almost any functionality or covalent label can be incorporated into anomeric DNAs by base modification without disturbing the helix structure. Clickable DNA can be used as hybridization probes in nucleic acids diagnostics, chemical biology, and material science to expand the toolbox of nucleic acid applications beyond canonical DNA.

Experimental Section

General: All chemicals and solvents were of laboratory grade as obtained from Acros Organics or Sigma Aldrich and were used without further purification. Flash column chromatography (FC): silica gel 60 from VWR (40–60 µM) at 0.4 bar. UV-spectra were recorded on a Hitachi U-3000 UV spectrophotometer: λmax (λ) in nm, ε in dm³ mol⁻¹ cm⁻¹. ¹³C NMR spectra were measured on a Varian AS 400 or AS 600 Mercuryplus or Bruker Autoflex 300 NMR spectrophotometer at 599.74, 399.89, or 300.15 MHz for 'H at 150.82, 100.56, or 75.47 MHz for 'C, and at 121.5 MHz for 'JC. H,¹³C correlated
(HMBC, HSQC) NMR spectra were used for the assignment of the $^{13}$C signals (Table S1). The $J$ values are given in Hz; $\delta$ values in ppm relative to Me$_4$Si as internal standard. For NMR spectra recorded in [D$_6$]DMSO, the chemical shift of the solvent peak was set to 2.50 ppm for $^1$H NMR and 39.50 ppm for $^{13}$C NMR. ESI-TOF mass spectra of nucleosides were recorded on a Micro-TOF spectrometer.

Oligonucleotide syntheses and characterization: Solid-phase oligonucleotide syntheses were performed on an ABI 392-08 synthesizer at 1 $\mu$mol scale (trityl-on mode) employing the phosphoramidites 4, 12, 15$^{10}$ and 16$^{11}$ as well as the standard building blocks with an average coupling yield over 95%. After cleavage from the solid support, the oligonucleotides were deprotected in 28% aqueous ammonia at 55°C for 2 h. The DMT-containing oligonucleotides were purified by reversed-phase HPLC (RP-18) with the gradient system at 260 nm: A) MeCN, B) 0.1 M (Et$_3$NH)OAc (pH 7.0)/MeCN, 95:5; gradient I: 0–3 min 10–15% A in B, 3–15 min 15–50% A in B; flow rate 0.7 mL/min. The purified “trityl-on” oligonucleotides were treated with 2.5% CHCl$_3$/COOH/CH$_2$Cl$_2$ for 2 min at 0°C to remove the 4,4’-dimethoxytrityl residues. The detritylated oligomers were purified again by reversed-phase HPLC with gradient II: 0–20 min 0–20% A in B, 20–25 min, 20% A in B; flow rate 0.7 mL/min. The oligonucleotides were desalted on a reversed-phase column using water for elution of salt, while the oligonucleotides were eluted with H$_2$O/CH$_3$OH (2:3). The oligonucleotides were lyophilized on a Speed-Vac evaporator to yield colorless solids which were frozen at −24°C. The molecular masses of the oligonucleotides were determined by MALDI-TOF mass spectrometry on a Bruker Autoflex Speed in the linear positive mode with 3-hydroxypicolinic acid (3-HPA) as a matrix. The thermal melting curves were measured with an Agilent Technologies Cary 100 Bio UV-vis spectrophotometer equipped with a thermo-electrical controller. The temperature was measured continuously in the reference cell with a Pt-100 resistor with a heating rate of 1°C min$^{-1}$. Tm values were determined from the melting curves using the software Meltwin, version 3.0$^{12}$ CD spectra were recorded at 25°C on a Jasco J-815 spectrometer.

The extinction coefficients $\varepsilon$$_{260}$ of the nucleosides are: dA 15 400, dG 11 700, dT 8800, dC 7300, $\alpha$-5-octadynyl-dU (2) 3200, $\beta$-5-
1H mixture was stirred at RT under Ar until the starting material was (286 mg, 2.82 mmol), and octa-1,7-diyne (1.5 g, 14.1 mmol). The 1-(2-Deoxy-5-(100 mg, 0.3 mmol) was dried by repeated co-evaporation with dry 4.27–3.99 (m, 1H, H-4) 2CH(CD3)2) 230 (11 900), 293 17.3 (CH3); UV (MeOH): λmax (α) = 233 (31000), 284 (11 500 mol−1 dm−3 cm−1); HRMS (ESI-TOF): m/z calcd for C16HsN3NaO4+: 657.2577 [M+Na]+; found: 657.2572.

1-(2-Deoxy-α-D-erythro-pentofuranosyl)-5-(octa-1,7-dinylyl)uracil 3 (2- cyanoyl)-N4-diisopropylphosphoramide (4): To a solution of compound 3 (100 mg, 0.16 mmol) and anhydrous iPr2EtN (53 µL, 0.8 mmol) in anhydrous CHCl3 (8.0 mL), 2-cyanoyl diisopropylphosphoramic chloride (47 µL, 0.27 mmol) was added at RT. After stirring for 20 min, the mixture was diluted with CHCl3 (15 mL) and the reaction was quenched by adding 5% aq. NaHCO3 solution (25 mL). Then, the aqueous layer was extracted with CHCl3 (60 mL), the combined organic layer was dried (Na2SO4) and evaporated. The residual colorless amyl was applied to FC (silica gel, column 10×2 cm, CH2Cl2/acetone, 9:1). From the main zone a colorless foam of compound 4 was obtained as a mixture of diastereoisomers (90 mg, 68%). TLC (silica gel, CH2Cl2/acetone, 80:20) Rf = 0.4; 1P NMR (121 MHz, CDCl3, 26 °C): δ = 149.14; 149.37 ppm. HRMS (ESI-TOF) m/z [M+H]+ calcd for C16H18N4O10P: 857.3655; found 857.3654.

1-(2-Deoxy-α-D-erythro-pentofuranosyl)-5-(1-(pyren-1-ylmethyl)-1H,1,2,3-triazol-4-yl)hex-1-yn-1-yl)uracil 18: Compound 15 (50 mg, 0.15 mmol) and pyren methyl azide (54 mg, 0.21 mmol) were dissolved in THF/H2O (1:1, v/v, 4 mL), then sodium ascorbate (67 µL, 0.6 mmol) of a freshly prepared 1 M solution in water was added, followed by the addition of copper(II) sulfate pentahydrate 7.5% in water (53 µL, 0.015 mmol). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated, and the residue was purified by FC (silica gel, column 10×3 cm, CH2Cl2/MeOH, 25:1) to give 17 (54 mg, 62%) as a light yellow solid. Rf = 0.40 (CH2Cl2/MeOH, 10:1); 1H NMR (600 MHz, D2)DSMO, 26 °C): δ = 11.52 (1H, NH, 8.51 (d, J = 9.3 Hz, 1H, arom. H), 8.36–8.27 (m, 4H, arom. H), 8.23–8.17 (m, 2H, arom. H), 8.10 (t, J = 7.6 Hz, 1H, arom. H), 8.04 (s, 1H, H-6), 7.98 (d, J = 7.9 Hz, 1H, ar), 7.92 (s, 1H, C=C), 6.32 (2H, CH2), 6.10 (dd, J = 7.7, 2.3 Hz, 1H, H-1), 5.37 (d, J = 2.9 Hz, 1H, H-3), 4.86 (t, J = 5.6 Hz, 1H, arom. H), 4.23 (dd, J = 4.4, 2.8, 1.5 Hz, 1H, H-3); 4.18 (td, J = 4.8, 1.6 Hz, 1H, H-4), 3.37 (t, J = 5.1 Hz, 2H, H-5), 2.60 (t, J = 7.5 Hz, 2H, CH2), 2.58–2.52 (m, 1H, H-2), 2.35 (tt, J = 14.0, 7.8 Hz, 1H, H-1), 1.55 (dd, J = 14.4, 2.1 Hz, 1H, H-2)., 1.66 (dd, J = 9.2, 7.6 Hz, 2H, CH3), 1.49 (dq, J = 9.6, 7.2 Hz, 2H, CH3); 13C NMR (151 MHz, D2DSMO, 26 °C): δ = 161.0 (C-6), 149.6 (C-2), 149.6 (Ar-C), 134.6 (C-3), 130.9 (1-azoce-103872 (14 of 16) © 2021 The Authors. Chemistry - A European Journal published by Wiley-VCH GmbH
7.2-Deoxy-5-O-(4,4'-dimethoxytrityl)-O-α-erythro-erythopentofuranosyl-4-(isobutylamino)-5-(octa-1,7-diylyl)-7H-pyrrolo[2,3-d]pyrimidine (11): Compound 10 (280 mg 0.66 mmol) was dissolved in anhydrous pyridine (5 mL) and treated with 4,4'-dimethoxytrityl chloride (291 mg, 0.86 mmol). The reaction mixture was stirred for 1 h at RT. Then, CHCl₃ (20 mL) was added and the org. layer was washed with 5% NaHCO₃ (35 mL). The org. phase was dried over Na₂SO₄ filtrated and evaporated. The remaining residue was applied to FC (silica gel, column 12×3 cm, CHCl₃/aceton, 85:15). From the main zone 11 was obtained as colorless foam (238 mg, 49%). TLC (CH₃Cl/acetone, 85:15): Rₜ = 0.50. 1H NMR (600 MHz, D₂O, 26°C): δ = 9.97 (s, 1H, NH), 8.62 (s, 1H, H-2'), 8.10 (s, 1H, H-6'), 7.37–7.42 (m, 2H, 2x CH₂), 7.25–7.29 (m, 4H, arom. H), 6.88–6.93 (m, 4H, arom. H), 6.71 (dd, J = 7.9, 3.3 Hz, H-1'), 5.60 (dd, J = 3.9, 1.1 Hz, H-1, HO), 4.28 (m, 2H, H-3, H-4'), 3.12 (dd, J = 10.2, 3.8 Hz, 1H, H-5'), 3.01 (dd, J = 10.2, 4.9 Hz, 1H, H-5). 13C NMR (125 MHz, D₂O, 26°C): δ = 175.6 (C-11), 158.1 (Ar-11), 151.4 (C-16), 151.3 (C-4), 150.9 (C-2), 144.8 (Ar-13), 135.6 (Ar-13), 135.5 (Ar-13), 129.3 (Ar-12), 129.7 (Ar-12), 127.9 (Ar-12), 127.7 (Ar-12), 126.7 (Ar-11), 113.3 (Ar-12), 112.8 (Ar-11), 110.3 (C-5), 96.7 (C-7), 91.3 (C-δ), 86.5 (CQ), 84.5 (C-4), 84.2 (C-3), 83.3 (C-11), 73.8 (C-13), 71.4 (C-3C), 64.0 (C-5C), 55.0 (CH₂), 39.8 (C-2), 34.5 (MeC), 27.3 (CH₂), 27.2 (CH₂), 19.12 (Me), 19.10 (Me), 18.6 (CH₃), 17.3 (CH₃); UV (MeOH): λₑₜₐₜ = 236 (37300), 276 (11300 mol⁻¹ dm³ cm⁻¹); HRMS (ESI-TOF): m/z calcd for C₂₅H₂₄N₄O₇+: 479.3315 [M+Na]+; found: 479.3309.

General procedure for Huisgen-Meldal-Sharpless [3+2] cycloaddition performed on oligo nucleotides in aqueous solution with 1-azidoethylphosphate: To a s-oligo nucleotide (5 µmol units) were added CuSO₄·TBA·2 H₂O (50 µmol), disodium hydrogen phosphate (120 µmol), and L-lysine hydrochloride (120 µmol) and stirred for 15 min at 50°C. The reaction mixture was dialyzed against 10 mM NaCl (50 mL), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in a speed-vac and dissolved in 500 µL bi-distilled water and centrifuged for 30 min at 14000 rpm. The supernatant solution was collected and further purified by reversed-phase HPLC with the gradient 0–3 min 10–15% B in A, 3–15 min 15–50% B in A, 15–20 min 50–10% B in A, flow rate 0.7 cm/min⁻¹. The molecular masses of the oligo nucleotides were determined by MALDI-TOF spectra (Table 1).
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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: anomeric DNA · chirality · click chemistry · hybridization · oligonucleotides

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