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Double-Headed Nucleotides with Arabino Configuration: Synthesis and Hybridization Properties

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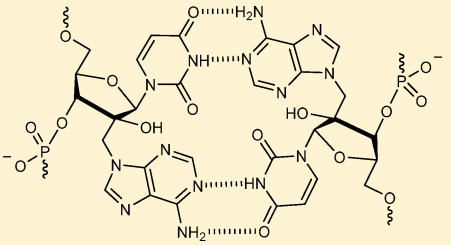
Supporting Information

ABSTRACT: The formation of new nucleic acid motifs by using double-headed nucleotides is reported. Modified phosphoramidites carrying additional thymine or adenine attached to the 2′-position of arabinouridine through a methylene linker are conveniently prepared and incorporated into oligonucleotides to obtain the modified nucleotide monomers $^{a}\mathbf{U}_{\mathbf{T}}$ and $^{a}\mathbf{U}_{\mathbf{A}}$, respectively. The extension of a DNA double helix by one or two additional A:T base pairs is achieved by placing these modified monomers in the opposite strands in a so-called (+1)-zipper arrangement. Hence, 12 basepairs can be presented in an 11-mer or even a 10-mer duplex. The modified nucleotide monomers also behave as dinucleotides when base-paired with two complementary nucleotides from the opposite strand. A new nucleic acid motif is introduced when two $^{a}\mathbf{U}_{\mathbf{A}}$ monomers recognize each other in the center of a duplex.



INTRODUCTION

Originally only seen as a genetic material, DNA has found a wide range of applications in DNA nanotechnology by virtue of its unique property to self-assemble into predictable structures through Watson−Crick base pairing between two complementary polynucleotide chains.[1−3] In combination with automated solid-phase DNA synthesis, various functional materials have been constructed by using chemically modified nucleotide monomers.[4−6] We and others have been investigating nucleotides with two nucleobases attached to the same sugar unit, commonly termed as double-headed nucleotides, with the purpose of exploiting the base pairing properties of the additional nucleobases.[7−23] Recently, we introduced a double-headed nucleotide monomer ($\mathbf{U}_{\mathbf{T}}$, Figure 1) with an ability to basepair with two adenines from the complementary strand.[18] Thus, the additional thymine in the 2′-position of the 2′-deoxyuridine attached through a methylene linker recognizes the opposite adenine at the center of a dsDNA. The fidelity of this recognition was also established as the additional thymine successfully discriminates the opposite mismatch nucleotide, though to a lesser extent when the opposite nucleobase was guanine.[18] The scope of this recognition was further extended with the introduction of the monomer $\mathbf{U}_{\mathbf{A}}$ (Figure 1) carrying adenine as the additional nucleobase.[20] The additional adenine was also found to basepair with a thymine from the opposite strand, and a similar behavior for all 16-possible base combinations can be argued.[20] Furthermore, formation of a specific Watson−Crick A:T base pair between the additional bases from monomers $\mathbf{U}_{\mathbf{T}}$ and $\mathbf{U}_{\mathbf{A}}$ in a 5′-$\mathbf{U}_{\mathbf{T}}$A:3′-A$\mathbf{U}_{\mathbf{A}}$ motif was established, and the modified duplexes were found to be thermally more stable compared to the unmodified duplexes.[20] In others words, the DNA double helix was extended by an additional base pair on the same backbone. These results provided motivation for further studies, for instance, of other base combinations, and elaborated structural studies or polymerization studies. However, the tedious and low-yielding synthesis (4.4% over 11 steps for the phosphoramidite of $\mathbf{U}_{\mathbf{T}}$ and 0.7% over 12 steps for the phosphoramidite of $\mathbf{U}_{\mathbf{A}}$, starting from ribose) posed a major

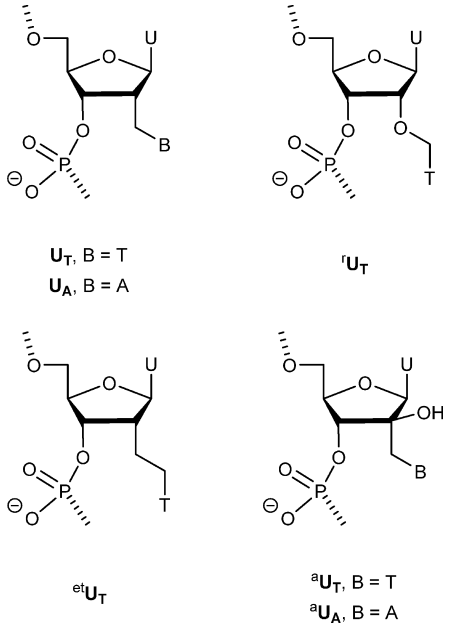


Figure 1. Double-headed nucleotides. U = uracil-1-yl, T = thymin-1-yl, A = adenin-9-yl.

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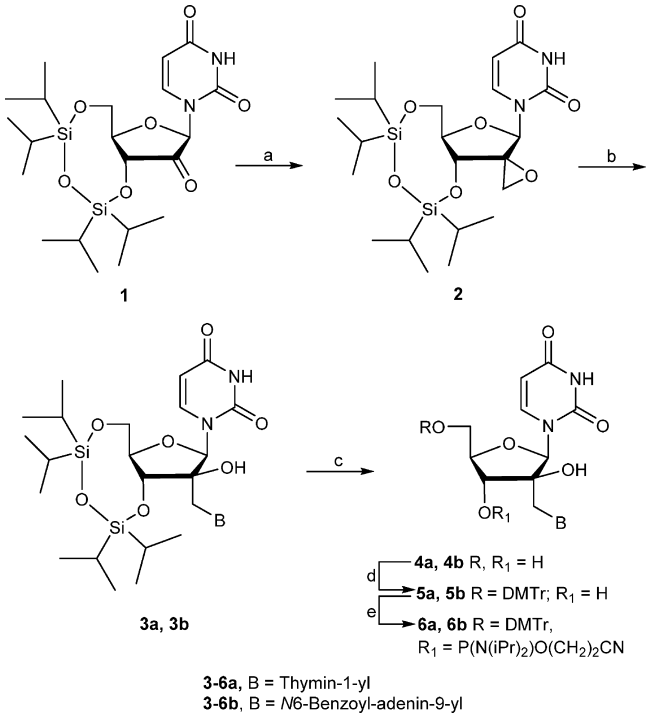
challenge and motivated us to find alternative double-headed nucleotides that could be significantly easier to synthesize yet have properties similar to those of the monomers $\mathbf{U}_T$ and $\mathbf{U}_A$. In this regard, we have recently presented a closely related double-headed nucleotide monomer ($^{t}\mathbf{U}_T$, Figure 1) based on uridine with a methylene linker between an additional thymine and the 2′-*O*-position.[21] Although efficiently prepared from uridine in few steps, this monomer was found to be much inferior to $\mathbf{U}_T$ in terms of basepairing with two adenines from the opposite strand, probably due to the longer and more flexible oxymethylene linker.[21] In a previous study, an analogue with an ethylene linker ($^{et}\mathbf{U}_T$, Figure 1) has also been shown not to involve the additional thymine in the base-pairing.[9] In combination, these studies suggested that a short methylene linker between the 2′-position and the additional base, as well as the 2′-endo conformation, is crucial to harness the base-pairing properties from the additional nucleobase in the core of duplex. We therefore envisioned that double-headed nucleosides based on an arabino configuration would be optimal. These would adopt 2′-endo conformations in parallel to the 2′-deoxy nucleosides and form B-type duplexes on hybridization with DNA complements. Therefore, we hereby introduce the monomers $^{a}\mathbf{U}_T$ and $^{a}\mathbf{U}_A$ (Figure 1) as analogues of $\mathbf{U}_T$ and $\mathbf{U}_A$. Easy access to these modified monomers from uridine is envisioned, and their potential to involve the additional nucleobase in the Watson−Crick base pairing is studied.

RESULTS AND DISCUSSION

Chemical Synthesis. The synthetic pathway to introduce the modified nucleotide monomers ($^{a}\mathbf{U}_T$, and $^{a}\mathbf{U}_A$) is shown in Scheme 1. First, uridine was conveniently converted to the well-known ketone **1**[24] and then to the spiro nucleoside **2**[25] in three high-yielding steps. Hereafter, the epoxide ring from **2** was opened by using thymine or 6*N*-benzoyladenine to obtain protected double-headed nucleosides **3a** and **3b**, respectively. *N*9-Alkylation of the adenine was confirmed by the presence of $^{3}J_{CH}$ coupling between the 2′-methylene protons and both C8 and C4 of the adenine in the HMBC NMR spectrum of **3b**. Similarly, a $^{3}J_{CH}$ coupling between the 2′-methylene protons and C2 as well C6 of the thymine in the HMBC NMR spectrum of **3a** ascertained *N*1-alkylation. Deprotection using TBAF afforded the double-headed nucleosides **4a** and **4b**. Coupling between H1′ and methylene protons in the ROESY spectra of **4a** and **4b** confirmed the desired stereochemistry. 5′-*O*-Dimethoxytritylation to give **5a** and **5b** followed by 3′-*O*-phosphitylation using standard procedures afforded the modified phosphoramidites **6a** and **6b**, respectively. The identity of all of the new compounds was fully ascertained by NMR spectroscopy (1H, ^{13}C, ^{31}P, COSY and HSQC) and HRMS. The modified phosphoramidites **6a** and **6b** were then used as starting material to introduce the modified nucleotide monomers ($^{a}\mathbf{U}_T$ and $^{a}\mathbf{U}_A$, respectively) into oligonucleotides by using standard solid-phase DNA synthesis. Standard conditions using tetrazole as the activator for the coupling reaction were used, and >80% coupling yields were obtained. Careful deprotection using concentrated ammonia at room temperature for 24 h was applied, as longer periods or elevated temperatures led to smaller isolated yields and impurities in the oligonucleotides.

Scheme 1[a]



[a]Reagents and conditions: (a) NaH, $(CH_3)_3SOI$, THF, DMSO, 89%; (b) **3a**: NaH, thymine, DMF, 53%; **3b**: KHMDS, *N*6-benzoyladenine, THF, 62%; (c) TBAF, THF, 87% **4a**, 76% **4b**; (d) DMTCl, pyridine, 81% **5a**, 70% **5b**; (e) $NC(CH_2)_2OP(N(i\text{-}Pr)_2)Cl$, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 72% **6a**, 76% **6b**. DMTr = 4,4′-dimethoxytrityl.

Hybridization Studies. First, the effect of single incorporations of the monomers $^{a}\mathbf{U}_T$ and $^{a}\mathbf{U}_A$ was studied by introducing these monomers at various positions in an 11-mer duplex (Table 1). The melting temperatures (T_m) of the resulting duplexes were derived from the UV melting curves at neutral pH 7 (Table 1) and compared with T_m of the corresponding unmodified duplex (Table 1, B = T, T_m = 44.0 °C), and the differences in melting temperature (ΔT_m's) were determined (Table 1, entries 1−5). For a direct comparison, T_m values for the corresponding 2′-deoxyuridine analogues ($\mathbf{U}_T$ and $\mathbf{U}_A$)[18,20] are also given (Table 1).[26] The T_m values of the modified duplexes were found to be consistently lower than the unmodified duplex (Table 1). A single incorporation of the monomer $^{a}\mathbf{U}_T$ or $^{a}\mathbf{U}_A$ led to a decrease in the thermal stability of the modified duplexes by 7.5−14.5 °C and 3.0−10.0 °C, respectively (Table 1). However, when compared to the similar duplexes with $\mathbf{U}_T$ and $\mathbf{U}_A$, the T_m values are in general strikingly similar. Hence, an additional thermal penalty of 0−1.5 °C was observed for the monomer $^{a}\mathbf{U}_T$ compared to the monomer $\mathbf{U}_T$ in the studied duplexes (compare $^{a}\mathbf{U}_T$ with $\mathbf{U}_T$, entries 1−5), whereas the thermal stability of the modified duplexes carrying the monomer $^{a}\mathbf{U}_A$ was found to be higher by 1.5−3.5 °C compared to the duplexes modified with $\mathbf{U}_A$ (compare $^{a}\mathbf{U}_A$ with $\mathbf{U}_A$ entries 1−5). Overall, these data show that the extra hydroxyl group from $^{a}\mathbf{U}_T$ and $^{a}\mathbf{U}_A$ does not lead to any major changes in duplex stability and is well tolerated in the modified duplexes.

Hereafter, the formation of an additional A:T base pair between the additional thymine and the additional adenine from the modified monomers $^{a}\mathbf{U}_T$ and $^{a}\mathbf{U}_A$, respectively, was studied. For this, monomers $^{a}\mathbf{U}_T$ and/or $^{a}\mathbf{U}_A$ were placed in a so-called (+1)-zipper arrangement in the center of an 11-mer DNA duplex (Table 2). The duplex featuring a 5′-$^{a}\mathbf{U}_A$A:3′-A$^{a}\mathbf{U}_T$ motif (entry 3) displayed an increase of 6.5 °C in the duplex thermal stability compared to the unmodified 11-mer duplex (Table 2, entry 1). When compared to the corresponding regular 12-mer duplex (5′-UAA:3′-ATU motif, entry 2), an increase of 6.0 °C in the

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Table 1. Hybridization Data for the Duplexes with Single Modification

entry	duplex	B =	T_m [a] (ΔT_m [b]/mod)/°C U_T	^aU_T	U_A	^aU_A
1	5'-d(CGC ATA **B**TC GC) 3'-d(GCG TAT AAG CG)		37.0 (−7.0)	35.5 (−8.5)	34.0 (−10.0)	36.0 (−8.0)
2	5'-d(CGC A**B**A TTC GC) 3'-d(GCG TAT AAG CG)		38.0 (−6.0)	36.5 (−7.5)	39.5 (−4.5)	41.0 (−3.0)
3	5'-d(CGC ATA T**B**C GC) 3'-d(GCG TAT AAG CG)		34.0 (−10.0)	34.0 (−10.0)	nd	34.0 (−10.0)
4	5'-d(CGC ATA TTC GC) 3'-d(GCG TA**B** AAG CG)		37.0 (−7.0)	35.5 (−8.5)	38.5 (−5.5)	40.0 (−4.0)
5	5'-d(CGC ATA TTC GC) 3'-d(GCG **B**AT AAG CG)		29.5 (−14.5)	29.5 (−14.5)	36.0 (−8.0)	39.5 (−4.5)

[a]Melting temperatures (T_m's) obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in a buffer containing 2.5 mM Na_2HPO_4, 5.0 mM NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0 using 1.0 μM concentrations of each strand. All determinations are averages of at least duplicates within 0.5 °C. [b]ΔT_m = change in T_m's relative to the unmodified reference duplex; **B** = T, T_m = 44.0 °C.

Table 2. Hybridization Data for the Duplexes with Modification in Both Strands

entry	duplex	T_m [a]	duplex	T_m [a]
1	5'-d(CGC ATA TTC GC) 3'-d(GCG TAT AAG CG)	44.0		
2	5'-d(CGC AUAA TTC GC) 3'-d(GCG TATU AAG CG)	44.5		
3	5'-d(CGC A^a**U$_A$**A TTC GC) 3'-d(GCG TA^a**U$_T$** AAG CG)	50.5	5'-d(CGC A**U$_A$**A TTC GC) 3'-d(GCG TA**U$_T$** AAG CG)	51.0
4	5'-d(CGC AUTA TTC GC) 3'-d(GCG TAAU AAG CG)	45.0		
5	5'-d(CGC A^a**U$_T$**A TTC GC) 3'-d(GCG TA^a**U$_A$** AAGCG)	51.5	5'-d(CGC A**U$_T$**A TTC GC) 3'-d(GCG TA**U$_A$** AAG CG)	50.0
6	5'-d(CGC A^a**U$_A$**A TTC GC) 3'-d(GCG TA^a**U$_A$** AAG CG)	40.5	5'-d(CGC A**U$_A$**A TTC GC) 3'-d(GCG TA**U$_A$** AAGCG)	40.5
7	5'-d(CGC A^a**U$_T$**A TTC GC) 3'-d(GCG TA^a**U$_T$** AAGCG)	42.5	5'-d(CGC A**U$_T$**A TTC GC) 3'-d(GCG TA**U$_T$** AAG CG)	44.0
8	5'-d(CGC AUAA TTC GC) 3'-d(GCG TAAU AAG CG)	33.0		
9	5'-d(CGC AUTA TTC GC) 3'-d(GCG TATU AAG CG)	34.0		
10	5'-d(CGC UAA UA A CGC) 3'-d(GCG ATU ATU GCG)	40.0		
11	5'-d(CGC ^{a}**U$_A$**A ^{a}**U$_A$** A CGC) 3'-d(GCG A^a**U$_T$** A^a**U$_T$** GCG)	47.5		
12	5'-d(GCT CAC UAA CTC CCA) 3'-d(CGA GTG ATU GAG GGT)	51.0		
13	5'-d(GCT CAC ^{a}**U$_A$**A CTC CCA) 3'-d(CGA GTG A^a**U$_T$** GAG GGT)	54.5		

[a]See Table 1. U corresponds to the incorporation of 2′-deoxyuridine.

duplex stability is seen (compare entry 3 with 2). A similar increase in the duplex stability was observed for the opposite arrangement of the motif (entry 5). The T_m value for this arrangement was found to be 7.5 and 6.5 °C higher than T_m values of the unmodified 11-mer duplex and the 12-mer duplex (5′-UTA:3′-AAU motif, entry 4), respectively. These data strongly suggest the formation of an additional A:T base-pair at the center of the modified duplexes. Furthermore, the thermal stability of the modified duplexes was comparable to the duplexes carrying the analogue 2′-deoxy monomers **U$_T$** and **U$_A$** in a similar context (entries 3 and 5).[26] Next, the specificity of this recognition is studied by introducing either of the monomer ^{a}**U$_T$** or ^{a}**U$_A$** in a (+1) zipper arrangement (5′-^{a}**U$_A$**A:3′-A^a**U$_A$**, and 5′-^{a}**U$_T$**A:3′A^a**U$_T$** motifs, entries 6 and 7, respectively). In this way, we introduced an A:A and a T:T mismatch with respect to the additional A:T base pair. The T_m's of these modified duplexes carrying a mismatch were found to be lower by 8.0−11.0 °C than the duplexes with the additional A:T base pair (compare entries 6 and 7 with either of 3 or 5). The magnitude of this discrimination is comparable to what was observed for duplexes modified with monomers **U$_T$** and **U$_A$** (a decrease of 6.0−10.5 °C) and for unmodified duplexes (a decrease of 10.5−12.0 °C, compare entries 8 and 9 with either of 2 or 4). These data suggest that the

Table 3. Hybridization Data (Dinucleotide Behavior)

entry	duplex	T_m [a]	duplex	T_m [a]
1	5'-d(GCT CAC U CTC CCA) 3'-d(CGA GTG A GAG GGT)	50.0		
2	5'-d(GCT CAC ^{a}**U$_A$** CTC CCA) 3'-d(CGA GTG A GAG GGT)	44.0	5'-d(GCT CAC **U$_A$** CTC CCA) 3'-d(CGA GTG A GAG GGT)	43.5
3	5'-d(GCT CAC ^{a}**U$_T$** CTC CCA) 3'-d(CGA GTG A GAG GGT)	42.5	5'-d(GCT CAC **U$_T$** CTC CCA) 3'-d(CGA GTG A GAG GGT)	44.5
4	5'-d(GCT CAC UA CTC CCA) 3'-d(CGA GTG AT GAG GGT)	51.0		
5	5'-d(GCT CAC ^{a}**U$_A$** CTC CCA) 3'-d(CGA GTG AT GAG GGT)	48.5	5'-d(GCT CAC **U$_A$** CTC CCA) 3'-d(CGA GTG AT GAG GGT)	50.0
6	5'-d(GCT CAC UA CTC CCA) 3'-d(CGA GTG AX GAG GGT)	42.5/46.5/42.0 X = C/G/A		
7	5'-d(GCT CAC ^{a}**U$_A$** CTC CCA) 3'-d(CGA GTG AX GAG GGT)	43.5/47.5/40.5 X = C/G/A	5'-d(GCT CAC **U$_A$** CTC CCA) 3'-d(CGA GTG AX GAG GGT)	41.5/49.0/42.0 X = C/G/A
8	5'-d(GCT CAC UT CTC CCA) 3'-d(CGA GTG AA GAG GGT)	51.5		
9	5'-d(GCT CAC ^{a}**U$_T$** CTC CCA) 3'-d(CGA GTG AA GAG GGT)	48.0	5'-d(GCT CAC **U$_T$** CTC CCA) 3'-d(CGA GTG AA GAG GGT)	50.5
10	5'-d(GCT CAC UT CTC CCA) 3'-d(CGA GTG AX GAG GGT)	42.0/40.5/45.0 X = T/C/G		
11	5'-d(GCT CAC ^{a}**U$_T$** CTC CCA) 3'-d(CGA GTG AX GAG GGT)	42.0/39.0/46.5 X = T/C/G	5'-d(GCT CAC **U$_T$** CTC CCA) 3'-d(CGA GTG AX GAG GGT)	44.5/42.0/49.0 X = T/C/G
12	5'-d(GCT CAC U CTC CCA) 3'-d(CGA GTG AT GAG GGT)	40.0		
13	5'-d(GCT CAC U CTC CCA) 3'-d(CGA GTG AA GAG GGT)	41.0		

[a]See Tables 1 and 2.

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presence of the hydroxyl group is not interfering with the base-pairing between the additional bases.

In addition, a 10-mer duplex with a 5′-$\mathbf{U_A}$A$\mathbf{U_A}$A:3′-A$\mathbf{U_T}$A$\mathbf{U_T}$ motif was studied with the aim of repeating the formation of the additional base-pair within the same duplex. Indeed, a stable duplex with T_m of 47.5 °C was obtained, which is 7.5 °C higher than for the unmodified 12-mer (compare entries 10 and 11). Thus, a short, stable 10-mer duplex with 12 base pairs was obtained. Finally, the formation of the additional A:T base pair was established in another sequence context (entry 13). The thermal stability of this modified 14-mer duplex was found to be 3.5 °C higher than the unmodified 15-mer duplex (compare entries 12 and 13). These data indicated that the formation of the additional base pair is not restricted to one sequence context and has a wider scope.

We then set out to validate the potential of the modified monomers ($^a\mathbf{U_A}$ and $^a\mathbf{U_T}$) to base pair with two nucleotides from the complementary strand. Their ability to behave as a compressed dinucleotide in parallel to what was found for the monomers $\mathbf{U_A}$ and $\mathbf{U_T}$ is discussed here. For this, two 13-mer ON sequences featuring either of the modified monomers ($^a\mathbf{U_A}$ and $^a\mathbf{U_T}$) in the center were prepared and mixed with different target DNA sequences (Table 3). When placed against one adenosine (5′-$^a\mathbf{U_A}$:3′A and 5′-$^a\mathbf{U_T}$:3′A motifs, entries 2 and 3), the modified monomers $^a\mathbf{U_A}$ and $^a\mathbf{U_T}$ induced a decrease of 6.0 and 7.5 °C, respectively, in the thermal stability compared to the regular 13-mer duplex (entry 1). This observation corroborates with the destabilization induced by these modified monomers in the first sequence context (Table 1). A comparable decrease of 6.5 and 5.5 °C in the duplex stability has been observed for monomers $\mathbf{U_A}$ and $\mathbf{U_T}$, respectively (Table 3, entries 2 and 3).[26] Hereafter, the modified monomers $^a\mathbf{U_A}$ and $^a\mathbf{U_T}$ were placed against two complementary nucleotides (entries 5 and 9). For the monomer $^a\mathbf{U_A}$ (5′-$^a\mathbf{U_A}$:3′AT motif, entry 5), a decrease of only 2.5 °C in the duplex stability compared to the unmodified 14-mer duplex (entry 4) was observed. However, compared to the unmodified T-bulge duplex (5′-U:3′AT motif, entry 12), the modified duplex was found to be 8.5 °C more stable (compare entries 5 and 12). A decrease of 3.5 °C was observed for the monomer $^a\mathbf{U_T}$ in a 5′-$^a\mathbf{U_T}$:3′AT motif compared to the unmodified duplex (compare entries 8 and 9). Nevertheless, the thermal stability of the modified duplex was found to be 7.0 °C higher than the A-bulged duplex (5′-U:3′AT motif, compare entries 9 and 13). These data strongly indicated base pairing between the additional nucleobases of the modified monomers $^a\mathbf{U_A}$ or $^a\mathbf{U_T}$ with the complementary nucleotide from the opposite strand in parallel to what was seen for the modified monomers $\mathbf{U_A}$ or $\mathbf{U_T}$. The decrease in T_m going from $\mathbf{U_T}$/$\mathbf{U_A}$ to $^a\mathbf{U_A}$/$^a\mathbf{U_T}$ is 1.5 and 2.5 °C, respectively (entries 5 and 9). Hereafter, the fidelity of this recognition was tested by changing the nucleotide opposite to the additional nucleobase (entries 7 and 11). A decrease of 1.0−8.0 °C in the duplex stability was observed for duplexes carrying a mismatch nucleotide opposite to the additional adenine from the monomer $^a\mathbf{U_A}$ with guanine as the least discriminated base. However, this discrimination is parallel to what was observed for the corresponding unmodified duplexes (a decrease of 4.5−9.0 °C) and duplexes modified with $\mathbf{U_A}$ (a decrease of 1.0−8.5 °C). On similar lines, duplexes with mismatch nucleotides against the additional thymine from $^a\mathbf{U_T}$ were found to be 1.5−9.0 °C less stable than the corresponding matched duplex. For regular thymidine in place of the additional thymine, mismatch duplexes were found to be 6.5−11.0 °C less stable and for the additional T in $\mathbf{U_T}$ 1.5−8.5 °C less stable. In general, duplexes carrying monomers $^a\mathbf{U_A}$ and $^a\mathbf{U_T}$ are around 1−4 °C less stable than the duplexes carrying 2′-deoxy analogues $\mathbf{U_A}$ and $\mathbf{U_T}$. Nevertheless, both $^a\mathbf{U_A}$ and $^a\mathbf{U_T}$ can basepair with two complementary nucleotides and can successfully discriminate between the bases opposite to the additional base with a potential similar to that for the monomers $\mathbf{U_A}$ and $\mathbf{U_T}$.

Importantly, the modified monomer $^a\mathbf{U_A}$ carries two complementary nucleobases on the same sugar unit. This design allowed us to test the potential of $^a\mathbf{U_A}$ to base pair with itself when placed in both strands of a DNA duplex (Table 4). The thermal stability of the modified duplex was determined and compared with that of the unmodified 14-mer duplex (Table 4, compare entries 1 and 4) as well as with that of the duplexes modified with the monomer $^a\mathbf{U_A}$ in either of the strands (entries 2 and 3). Duplexes featuring $^a\mathbf{U_A}$ against two complementary nucleotides (5′-$^a\mathbf{U_A}$:3′-AU motif and 5′-UA:3′ -$^a\mathbf{U_A}$) were found to be 2−3 °C less stable than the unmodified duplex. This observation is parallel to what we observed previously for 5′-$^a\mathbf{U_A}$:3′-AT (Table 3). Nevertheless, the duplex featuring $^a\mathbf{U_A}$ in both strands placed against each other was found to be 4.5 °C thermally more stable than the unmodified duplex (compare entry 4 with 1) demonstrating a stable base pairing between the modified monomers from opposite strands.

Table 4. Hybridization Data (Self-Pairing)

entry	duplex	T_m[a]
1	5′-d(GCT CAC UA CTC CCA) 3′-d(CGA GTG AU GAG GGT)	51.0
2	5′-d(GCT CAC $^a\mathbf{U_A}$ CTC CCA) 3′-d(CGA GTG AU GAG GGT)	48.0
3	5′-d(GCT CAC UA CTC CCA) 3′-d(CGA GTG $^a\mathbf{U_A}$ GAG GGT)	49.0
4	5′-d(GCT CAC $^a\mathbf{U_A}$ CTC CCA) 3′-d(CGA GTG $^a\mathbf{U_A}$ GAG GGT)	55.5

[a]See Tables 1 and 2.

CD Spectroscopy. The global structure of the modified duplexes was determined using CD spectroscopy. The CD spectra of all the modified duplexes display characteristic features of B-type duplex geometry, i.e., positive bands at around 220 and 280 nm and a negative band at around 250 nm (Figure 2 and Figures S7−S11, Supporting Information). In the CD spectra of duplexes carrying modified monomers ($^a\mathbf{U_T}$ and $^a\mathbf{U_A}$) in (+1)-zipper arrangement (in other words, containing additional base pairs) (Figure 2, entries 3 and 5, and Figure S9, Supporting Information), the bands at 250 and 280 nm are increased in intensity and the latter is moved to 275 nm as compared to the

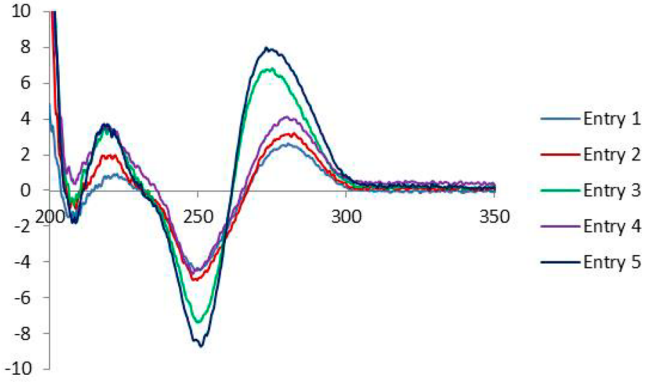

Figure 2. CD spectra of modified duplexes (referring to Table 2, entries 1−5).

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unmodified duplexes. However, similar changes in wavelength and intensity were seen previously for the duplexes with monomers $\mathbf{U_T}$ and/or $\mathbf{U_A}$ in similar arrangements.[20] No notable difference was observed in the CD spectra of duplexes with $^{a}\mathbf{U_T}$ or $^{a}\mathbf{U_A}$ behaving as dinucleotides (Figure S10, Supporting Information), which is also parallel to what was seen for $\mathbf{U_T}$ or $\mathbf{U_A}$.

Discussion. The two double-headed nucleotide monomers ($^{a}\mathbf{U_T}$ and $^{a}\mathbf{U_A}$) were very conveniently synthesized via the phosphoramidites **5a** and **5b**. The synthesis of **5a** and **5b** is achieved from uridine in only seven steps with overall yields of 21 and 19%, respectively. This is remarkably better than the synthesis of the phosphoramidites for $\mathbf{U_T}$ and $\mathbf{U_A}$ (overall yield of 4.4% over 11 steps, and 0.7% over 12 steps from ribose, respectively).[18,20] When studied in modified duplexes, the arabino-configured $^{a}\mathbf{U_T}$ and $^{a}\mathbf{U_A}$ demonstrated all the same features as the 2′-deoxyribo counterparts $\mathbf{U_T}$ and $\mathbf{U_A}$. Hence, when placed in a so-called (+1)-zipper arrangement, the additional adenine from $^{a}\mathbf{U_A}$ base pairs with the additional thymine from $^{a}\mathbf{U_T}$. In this way, an 11-mer DNA duplex carries 12 Watson−Crick base pairs. Mismatches (A:A or T:T) are also being discriminated. Both the modified monomers $^{a}\mathbf{U_T}$ and $^{a}\mathbf{U_A}$ also showed the capability to behave as compressed dinucleotides by basepairing to the two complementary nucleotides from the opposite strand. Thus, duplexes featuring the modified monomer ($^{a}\mathbf{U_A}$ or $^{a}\mathbf{U_T}$) against two complementary nucleotides showed only a small drop in the thermal stability when compared to the unmodified duplex. With sufficiently available arabino-configured amidites, more sequences can be studied. For instance, a stable 12-mer duplex carrying two additional base pairs was obtained by repeating the additional A:T base pair formed by $^{a}\mathbf{U_A}$ or $^{a}\mathbf{U_T}$ in the same duplex. Furthermore, the modified monomer $^{a}\mathbf{U_A}$ was also found to form base pair with itself when placed in opposite strands. This further consolidates the general structure of double-headed nucleotide structures with two bases in parallel orientation toward the duplex core for participating in the base pairing and information transfer in DNA. The 2′-OH group does not interfere with this as the hybridization behavior is remarkably similar to or without its presence as validated by both UV-melting studies and CD spectroscopy. In the future, we plan the preparation of monomers with different base combinations based on the general and easily available arabino-configured structure of $^{a}\mathbf{U_T}$ and $^{a}\mathbf{U_A}$.

■ CONCLUSION

Our hypothesis of obtaining similar properties by replacing the 2′-deoxyuridine sugar of our previously reported monomers $\mathbf{U_A}$ and $\mathbf{U_T}$ by arabino-uridine in monomers $^{a}\mathbf{U_A}$ and $^{a}\mathbf{U_T}$ is validated. Indeed, monomers $^{a}\mathbf{U_A}$ and $^{a}\mathbf{U_T}$ were obtained in remarkably improved yields over fewer steps than the 2′-deoxyuridine analogues ($\mathbf{U_A}$ and $\mathbf{U_T}$) and showed comparable results in terms of base-pairing properties involving additional nucleobases. Thus, the new monomers can find applications as new convenient tools in functional DNA nanotechnology.

■ EXPERIMENTAL SECTION

General Methods. All commercial reagents were used as supplied except CH_2Cl_2, which was distilled prior to use. Anhydrous solvents were dried over 4 Å activated molecular sieves (CH_2Cl_2, pyridine, and DCE) or 3 Å activated molecular sieves (DMF, CH_3CN). THF was freshly distilled over sodium, and anhydrous DMSO was obtained from commercial sources and used as such. Reactions were carried out under argon when anhydrous solvents were used. All reactions were monitored by TLC using silica gel plates (60 F_{254}). To visualize the plates, they were exposed to UV light (254 nm) and/or immersed in a solution of 5% H_2SO_4 in methanol (v/v) followed by charring. Column chromatography was performed with silica gel 60 (particle size 0.040−0.063 μm). Silica gel was pretreated with 1% pyridine in CH_2Cl_2 (v/v) for the purification of 4,4′-dimethoxytrityl-protected nucleosides. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded at 400, 101, and 162 MHz, respectively. Chemical shift values (δ) are reported in ppm relative to either tetramethylsilane (^{1}H NMR) or the deuterated solvents as internal standard for ^{13}C NMR (δ: $CDCl_3$ 77.16 ppm, DMSO-d_6 39.52 ppm) and relative to 85% H_3PO_4 as external standard for ^{31}P NMR. 2D spectra ($^{1}H-^{1}H$ COSY and $^{1}H-^{13}C$ HSQC) have been used in assigning ^{1}H and ^{13}C NMR signals. High-resolution ESI (quadrupole) mass spectra were recorded in positive ion mode.

*3′,5′-O-(Tetraisopropyldisiloxane-1,3-diyl)uridine 2′(S)-Spiroepoxide (**2**).*[25] NaH (60% suspension in mineral oil, 0.354 g, 8.84 mmol) was mixed with dry petroleum ether (15 mL) under an argon atmosphere, and the mixture was stirred for 30 min, after which the petroleum ether was decanted off. Anhydrous DMSO (12 mL) and trimethylsulfoxonium iodide (2.94 g, 13.2 mmol) were added, and the mixture was stirred for 30 min under an argon atmosphere. The reaction flask was then cooled to 0−5 °C, and anhydrous THF (10 mL) was added. A solution of the ketone **1** (2.14 g, 4.42 mmol) in THF (10 mL) was added dropwise at 0−5 °C, and the mixture was stirred at the same temperature for 30 min. Water (15 mL) was added slowly at 0−5 °C followed by the addition of aqueous saturated ammonium chloride (15 mL) and ethyl acetate (50 mL). The phases were separated, and the aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash chromatography (0−60% ethyl acetate in petroleum ether) to yield **2** (1.97 g, 89%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H, NH), 7.44 (d, J = 8.1 Hz, 1H, H6), 6.20 (s, 1H, H1′), 5.74 (d, J = 8.1 Hz, 1H, H5), 4.48 (d, J = 9.0 Hz, 1H, H3′), 4.11 (dq, J = 13.1, 2.8 Hz, 2H, H5′), 3.89 (dt, J = 9.0, 2.8 Hz, 1H, H4′), 3.24 (d, J = 5.4 Hz, 1H, CH_{2a}), 3.00 (d, J = 5.4 Hz, 1H, CH_{2b}), 1.18−0.83 (m, 28H, $CH(CH_3)_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.4 (C4), 150.3 (C2), 140.1 (C6), 102.6 (C5), 81.2 (C1′), 80.8 (C4′), 68.6 (C3′), 65.4 (C2′), 60.7 (C5′), 49.2 (CH_2), 17.4, 17.3, 17.3, 17.0, 16.9, 16.8, 16.8 ($CH(CH_3)_2$), 13.3, 13.0, 12.7, 12.4 ($CH(CH_3)_2$); ESI HRMS m/z 499.2303 ($[M + H]^+$, $C_{22}H_{38}N_2O_7Si_2H^+$ calcd 499.2290).

*1-(2′-C-(Thymin-1-yl)methyl-3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)arabinofuranosyl)uracil (**3a**).* A mixture of vacuum-dried thymine (0.481 g, 3.81 mmol) and NaH (60% suspension in mineral oil, 53 mg, 1.33 mmol) in dry DMF (3 mL) was stirred under argon at room temperature for 1 h. A solution of **2** (0.475 g, 0.95 mmol) in dry DMF (3 mL) was added, and the mixture was stirred at 110 °C for 3 h. After the solution was cooled to room temperature, a saturated aqueous solution of ammonium chloride (3 mL) was added followed by water (15 mL) and ethyl acetate (10 mL). The aqueous phase was separated and extracted with ethyl acetate (3 × 5 mL). The combined organic phase was washed with brine (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was purified by flash chromatography (0−60% ethyl acetate in petroleum ether) to yield **3a** (0.316 g, 53%) as a white foam: R_f 0.4 (50% ethyl acetate in petroleum ether); ^{1}H NMR (400 MHz, DMSO) δ 11.37 (s, 1H, NH), 11.32 (d, J = 1.6 Hz, 1H, NH), 7.56 (s, 1H, H6(T)), 7.53 (d, J = 8.1 Hz, 1H, H6(U)), 5.90 (s, 1H, H1′), 5.89 (s, 1H, 2′-OH), 5.53 (dd, J = 8.1, 1.6 Hz, 1H, H5), 4.19−3.91 (m, 6H, H3′, H4′, 2 × H5′, 2′-CH_2), 1.74 (s, 3H, CH_3), 1.07−0.95 (m, 28H, $CH(CH_3)_2$); ^{13}C NMR (101 MHz, DMSO) δ 164.0 (C4(T)), 162.9 (C4(U)), 153.0 (C2(T)), 150.2 (C2(U)), 142.6 (C6(T)), 140.0 (C6(U)), 108.1 (C5(T)), 100.7 (C5(U)), 84.2 (C1′), 79.5 (C4′), 78.7 (C2′), 75.2 (C3′), 60.0 (C5′), 49.9 (2′-CH_2), 17.2, 17.1, 17.0, 16.8, 16.7, 16.7, 16.6 ($CH(CH_3)_2$), 12.7, 12.2, 12.0, 11.8 ($CH(CH_3)_2$), 12.1 (CH_3 (T)); ESI HRMS m/z 647.2529 ($[M + Na]^+$, $C_{27}H_{44}N_4O_9Si_2Na^+$ calcd 647.2539).

*1-(2′-C-(6N-Benzoyladenin-9-yl)methyl-3′,5′-O-(tetraisopropyldisiloxane-1,3-diyl)arabinofuranosyl)uracil (**3b**).* A 1 M solution of KHMDS in THF (3.2 mL, 3.2 mmol) was added dropwise to a

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suspension of 6*N*-benzoyladenine (1.00 g, 4.1 mmol) in dry THF (10 mL). The mixture was stirred at 55 °C for 1 h. A solution of **2** (1.00 g, 2.0 mmol) in dry THF (20 mL) was added dropwise, and the mixture was stirred at 55 °C for 24 h. After the mixture was cooled to room temperature, a saturated aqueous solution of ammonium chloride (50 mL) was added followed by water (50 mL) and CH_2Cl_2 (100 mL). The aqueous phase was separated and extracted with ethyl acetate (2 × 50 mL). The combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (0−2% MeOH in CH_2Cl_2) to afford **3b** (0.920 g, 62%) as a white foam: R_f 0.4 (3% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.66 (bs, 1H, NH(U)), 9.95 (s, 1H, NH(A)), 8.65 (s, 1H, H2(A)), 8.24 (s, 1H, H8(A)), 7.92 (d, J = 7.2 Hz, 2H, Bz), 7.52−7.42 (m, 4H, H6(U), Bz), 6.98 (bs, 1H, 2′-OH), 5.71 (s, 1H, H1′), 5.67 (d, J = 8.2 Hz, 1H, H5(U)), 4.70 (d, J = 15.4 Hz, 1H, 2′-CH_{2a}), 4.58 (d, J = 15.4 Hz, 1H, 2′-CH_{2b}), 4.42 (d, J = 9.0 Hz, 1H, H3′), 4.12 (d, J = 12.5 Hz, 1H, H5′), 4.04 (d, J = 12.5 Hz, 1H, H5′), 3.80 (d, J = 9.0 Hz, 1H, H4′), 1.10−1.04 (m, 28H, $CH(CH_3)_2$); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.8 (CO), 162.1 (C4(U)), 152.1 (C4(A)), 150.1 (C2(A)), 149.7, 149.5, (C6(A), C2(U)), 143.5 (C8(A)), 140.3 (C6(U)), 131.7, 131.7, 127.8, 127.5 (Bz), 123.7 (C5(A)), 101.0 (C5(U)), 83.7 (C1′), 78.7 (C4′), 76.2 (C2′), 75.9 (C3′), 59.1 (C5′), 49.3 (CH_2), 16.4, 16.3, 16.3, 16.2, 16.1, 16.0, 15.9, 15.9 ($CH(CH_3)_2$), 12.4, 11.9, 11.7, 11.4 ($CH(CH_3)_2$); ESI HRMS *m*/*z* 738.3038 ([M + Na]$^+$, $C_{34}H_{47}N_7O_8Si_2H^+$ calcd 738.3097).

*1-((2′-C-(Thymin-1-yl)methyl)arabinofuranosyl)uracil (**4a**).* To a solution of double-headed nucleoside **3a** (0.280 g, 0.45 mmol) in THF (10 mL) was added a 1 M solution of TBAF in THF (1.1 mL, 1.1 mmol), and the mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated at reduced pressure, and the residue was purified by column chromatography (0−10% MeOH in CH_2Cl_2) to obtain **4a** (150 mg, 87%) as white solid: R_f 0.3 (10% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 11.39 (s, 1H, NH), 11.29 (s, J = 2.0 Hz, 1H, NH), 7.71 (d, J = 8.0 Hz, 1H, H6(U)), 7.45 (d, J = 1.0 Hz, 1H, H6(T)), 5.95 (s, 1H, H1′), 5.86 (s, 1H, 2′-OH), 5.81 (d, J = 4.0 Hz, 1H, 3′-OH), 5.59 (dd, J = 8.0, 2.0 Hz, 1H, H5(U)), 5.38 (t, J = 5.2 Hz, 1H, 5′-OH), 3.93−3.83 (m, 4H, H3′, H4′, 2′-CH_2), 3.68−3.63 (m, 2H, 2 × H5′), 1.73 (d, 3H, J = 1.0 Hz, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 164.0 (C4(T)), 163.1 (C4(U)), 152.7 (C2(T)), 150.5 (C2(U)), 142.6 (C6(T)), 141.9 (C6(U)), 108.3 (C5(T)), 100.4 (C5(U)), 84.7 (C1′), 84.1 (C4′), 80.6 (C2′), 75.1 (C3′), 60.5 (C5′), 47.7 (2′-CH_2), 11.9 (CH_3); ESI HRMS *m*/*z* 405.0996 ([M + Na]$^+$, $C_{15}H_{18}N_4O_8Na^+$ calcd 405.1017).

*1-((2′-C-(6N-Benzoyladenin-9-yl)methyl)arabinofuranosyl)uracil (**4b**).* To a solution of double-headed nucleoside **3b** (0.50 g, 0.67 mmol) in THF (10 mL) was added a 1 M solution of TBAF in THF (1.8 mL, 1.8 mmol), and the mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated at reduced pressure, and the residue was purifed by column chromatography (0−10% MeOH in CH_2Cl_2) to obtain **4a** (255 mg, 76%) as white solid: R_f 0.3 (10% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 11.33 (s, 1H, NH), 11.17 (s, 1H, NH), 8.74 (s, 1H, H2(A)), 8.38 (s, 1H, H8(A)), 8.05 (d, J = 7.4 Hz, 2H, Bz), 7.69 (d, J = 8.0 Hz, 1H, H6(U)), 7.65 (t, J = 7.4 Hz, 1H, Bz), 7.55 (t, J = 7.4 Hz, 2H, Bz), 6.39 (d, J = 4.8 Hz, 1H, 3′-OH), 6.09 (s, 1H, H1′), 6.00 (s, 1H, 2′-OH), 5.63 (d, J = 8.0 Hz, 1H, H5(U)), 5.36 (t, J = 4.8 Hz, 1H, 5′-OH), 4.60 (d, J = 14.6 Hz, 1H, 2′-CH_2), 4.37 (d, J = 14.6 Hz, 1H, 2′-CH_2), 3.98 (m, 1H, H4′), 3.82 (m, 1H, H3′), 3.65−3.60 (m, 2H, 2 × H5′); ^{13}C NMR (101 MHz, DMSO) δ 165.3 (CO) 163.1 (C4(U)), 152.8 (C4(A)), 151.0, 150.6, 150.0 (C2(U), C2(A), C6(A)), 141.8 (C6(U)), 132.3, 131.0, 128.3 (Bz), 125.0 (C5(A))100.5 (C5(U)), 85.4, 85.3 (C4′, C1′), 80.7 (C2′), 75.2 (C3′), 60.8 (C5′), 44.4 (2′-CH_2); ESI HRMS: *m*/*z* 496.1561 ([M + H]$^+$, $C_{22}H_{21}N_7O_7H^+$ calcd 496.1575).

*1-((5′-O-(4,4′-Dimethoxytrityl)-2′-C-(thymin-1-yl)methyl)-arabinofuranosyl)uracil (**5a**).* Nucleoside **4a** (110 mg, 0.28 mmol) was coevaporated with anhydrous pyridine (2 × 5 mL) and redissolved in the same solvent (5 mL). DMTrCl (120 mg, 0.35 mmol) was added, and the reaction mixture was stirred at room temperature for 16 h. EtOH (99.9%, 2−3 drops) was added, and the mixture was concentrated under reduced pressure. The residue was redissolved in CH_2Cl_2 (20 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 × 15 mL). The combined aqueous phase was extracted with CH_2Cl_2 (2 × 15 mL), and the combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was coevaporated with a mixture of toluene and EtOH (20 mL 1:1, v/v) and purified by column chromatography (0−5% MeOH in CH_2Cl_2) to afford **5a** (160 mg, 81%) as a light yellow foam: R_f 0.4 (7% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 11.37 (s, 1H, NH), 11.32 (s, 1H, NH), 7.48 (d, J = 8.2 Hz, 1H, H6(U)), 7.41−7.39 (m, 3H, H6(T), DMTr), 7.33 (t, J = 7.6 Hz, 2H, DMTr), 7.27−7.22 (m, 5H, DMTr), 6.90 (d, J = 8.9 Hz, 4H, DMTr), 5.99 (s, 1H, H1′), 5.86 (d, J = 4.4 Hz, 1H, 3′-OH), 5.66 (s, 1H, 2′-OH), 5.39 (d, J = 8.2 Hz, 1H, H5(U)), 4.03−3.98 (m, 1H, H-4′), 3.87−3.82 (m, 3H, H3′, 2′-CH_2), 3.74 (s, 6H, 2 × OCH_3), 3.38−3.33 (m, 1H, H5′), 3.22 (dd, J = 10.3, 2.7 Hz, 1H, H5′), 1.71 (s, 3H, CH_3); ^{13}C NMR (101 MHz, DMSO) δ 164.0 (C4(T)), 162.9 (C4(U)), 158.0 (DMTr), 152.6 (C2(T)), 150.4 (C2(U)), 144.6 (DMTr), 142.4 (C6(T)), 141.7 (C6(U)), 135.4, 135.2, 129.6, 129.7, 127.7, 127.6, 126.6, 113.1 (DMTr), 108.3 (C5(T)), 100.3 (C5(U)), 85.4 (C2′), 84.8 (C1′), 82.5 (C4′), 80.3 (DMTr), 75.5 (C3′), 63.4 (C5′), 54.9 (OCH_3), 48.0 (2′-CH_2), 11.9 (CH_3); ESI HRMS *m*/*z* 707.2304 ([M + Na]$^+$, $C_{36}H_{36}N_4O_{10}Na^+$ calcd 707.2324).

*1-(2′-C-(6N-Benzoyladenin-9-yl)methyl-5′-O-(4,4′-dimethoxytrityl)arabinofuranosyl)uracil (**5b**).* Nucleoside **4b** (230 mg, 0.46 mmol) was coevaporated with anhydrous pyridine (2 × 5 mL) and redissolved in the same solvent (5 mL). DMTrCl (190 mg, 0.56 mmol) was added, and the reaction mixture was stirred at room temperature for 16 h. EtOH (99.9%, 2−3 drops) was added, and the mixture was concentrated under reduced pressure. The residue was redissolved in CH_2Cl_2 (30 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 × 20 mL). The combined aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic phase was dried (Na_2SO_4) and concentrated under reduced pressure. The residue was coevaporated with a mixture of toluene and EtOH (20 mL 1:1 v/v) and purified by column chromatography (0−5% MeOH in CH_2Cl_2) to afford **5b** (260 mg, 70%) as a light yellow solid: R_f 0.4 (7% MeOH in CH_2Cl_2); ^{1}H NMR (400 MHz, DMSO) δ 11.32 (s, 1H, NH), 11.15 (s, 1H, NH), 8.71 (s, 1H, H2(A)), 8.32 (s, 1H, H8(A)), 8.04 (d, J = 7.4 Hz, 2H, Bz), 7.64 (t,, J = 7.4 Hz, 1H, Bz), 7.55 (t,, J = 7.4 Hz, 2H, Bz),7.44 (d, J = 8.0 Hz, 1H, H6(U)), 7.39 (d, J = 7.6 Hz, 2H, DMTr), 7.32 (t, J = 7.6 Hz, 2H, DMTr), 7.26−7.22 (m, 5H, DMTr), 6.89 (d, J = 8.9 Hz, 4H, DMTr), 6.38 (d, J = 4.8 Hz, 1H, 3′-OH), 6.12 (s, 1H, H1′), 5.83 (s, 1H, 2′-OH), 5.45 (d, J = 8.0 Hz, 1H, H5(U)), 4.57 (d, J = 14.6 Hz, 1H, 2′-CH_2), 4.36 (d, J = 14.6 Hz, 1H, 2′-CH_2), 4.15−4.14 (m, 1H, H4′), 3.81−3.80 (m, 1H, H3′), 3.74 (s, 6H, 2 × OCH_3), 3.38−3.28 (m, 1H, H5′), 3.21 (dd, J = 9.8, 2.2 Hz, 1H, H5′); ^{13}C NMR (101 MHz, DMSO) δ 165.4 (CO), 163.0 (C4(U)), 158.1 158.0, (DMTr), 153.0 (C4(A)), 151.0 (C2(A)), 150.5 150.0 (C2(U), C6(A)), 145.5 (C8(A)), 144.6 (DMTr), 141.7 (C6(U)), 135.48, 135.40, 133.4, (DMTr), 132.3 (Bz), 129.76, 129.70 (DMTr), 128.4 (Bz), 127.9, 127.7, 126.7 (DMTr), 125.1 (C5(A)), 113.1(DMTr), 100.5 (C5(U)), 85.6 85.5 (DMTr, C1′), 83.7 (C4′), 80.4 (C2′), 75.6(C3′), 63.7 (C5′), 54.9 (OCH_3), 44.7 (2′-CH_2); ESI HRMS *m*/*z* 798.2850 ([M + H]$^+$, $C_{43}H_{39}N_7O_9H^+$ calcd 798.2882).

*1-((3′-O-(P-2-Cyanoethyl-N,N-diisopropylaminophosphinyl)-5′-O-(4,4′-dimethoxytrityl)-2′-C-(thymin-1-yl)methyl)arabinofuranosyl)uracil (**6a**).* Nucleoside **5a** (150 mg, 0.21 mmol) was coevaporated with DCE (2 × 5 mL) and redissolved in the same solvent (5 mL). *N*,*N*-Diisopropylchlorophosphoramidite (150 μL, 0.67 mmol) and DIPEA (190 μL, 1.09 mmol) were added, and the reaction mixture was stirred at room temperature for 16 h. EtOH (99.9%, 2−3 drops) was added, and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (0−5% MeOH CH_2Cl_2) to afford **6a** (140 mg, 72%) as a pale yellow solid: R_f 0.5 (3% MeOH in CH_2Cl_2); ^{31}P ($CDCl_3$) δ 152.4, 151.5; HR-ESI MS *m*/*z* 907.3379 ([M + Na]$^+$, $C_{45}H_{53}N_6O_{11}PNa^+$ calcd 907.3402).

*1-(2′-C-(6N-Benzoyladenin-9-yl)methyl-3′-O-(P-2-cyanoethyl-N,N-diisopropylaminophosphinyl)-5′-O-(4,4′-dimethoxytrityl)-arabinofuranosyl)uracil (**6b**).* Nucleoside **6a** (150 mg, 0.21 mmol) was coevaporated with DCE (2 × 5 mL) and redissolved in the same solvent (5 mL). *N*,*N*-Diisopropylchlorophosphoramidite (220 μL, 0.98 mmol) and DIPEA (320 μL, 1.83 mmol) were added, and the reaction mixture was stirred at room temperature for 16 h. EtOH (99.9%, 2−3 drops) was

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added, and the mixture was concentrated under reduced pressure. The residue was purified by column chromatography (0−5% MeOH CH_2Cl_2) to afford **6b** (220 mg, 76%) as a pale yellow solid: R_f 0.4 (5% MeOH in CH_2Cl_2); ^{31}P ($CDCl_3$) δ 152.0, 151.2; HR-ESI MS m/z 998.3938 ($[M + Na]^+$, $C_{52}H_{56}N_9O_{10}PH^+$ calcd. 998.3961).

Oligonucleotide Synthesis. The phosphoramidite approach was followed to prepare oligonucleotides using an automated DNA synthesizer. The modified phosphoramidites **6a** and **6b** were used to introduce monomers $^a\mathbf{U}_\mathbf{T}$ and $^a\mathbf{U}_\mathbf{A}$, respectively, and synthesis of modified oligonucleotides was performed on a 0.2 μmol scale (CPG support). The synthesis followed the regular protocol for the DNA synthesizer. For the modified phosphoramidites a prolonged coupling time of 20 min was used. 1*H*-Tetrazole was used as the activator. In general, coupling yields for all 2-cyanoethyl phosphoramidites were >80%. The 5′-*O*-DMT-ON oligonucleotides were removed from the solid support by treatment with concentrated aqueous ammonia at room temperature for 24 h, which also removed the protecting groups. The oligonucleotides were purified by reversed-phase HPLC on a Waters 600 system using a XBridge OST C18 column, 19 × 100 mm, 5 μm + precolumn: XBridge 10 × 10 mm, 5 μm, temperature 50 °C. Buffer A: 0.05 M triethylammonium acetate pH 7.4. Buffer B: MeCN/H_2O (3:1). Program used: 2 min 100% A, 100%−30%:0%−70% A:B over 17 min, 4 min 100% B, 6 min 100% A. Flow 5 mL/min. All fractions containing 5′-*O*-DMTr-protected oligonucleotide were collected and concentrated. The products were detritylated by treatment with 80% aqueous acetic acid for 30 min and neutralized by addition of sodium acetate (3 M, 15 μL), and then sodium perchlorate (5 M, 15 μL) was added followed by acetone (1 mL). The pure oligonucleotides precipitated overnight at −20 °C. The mixture was then placed in a centrifuge and subjected to 12000 rpm, 10 min at 4 °C. The supernatant was removed and the pellet washed with cold acetone (2 × 1 mL). The pellet was then dried for 30 min under reduced pressure and dissolved in pure water (1 mL) and the concentration measured as OD 260 nm. The extinction coefficients of the modified ON's were estimated from a standard method using micromolar extinction coefficients for the monomers. For the double-headed monomers, the extinction coefficients were assumed to equal to the sum of the two nucleobases. The purity and constitution of the ON's were confirmed by IC analysis and MALDI-TOF MS $[M - H]^+$, respectively (Table S1, Supporting Information).

Thermal Denaturation Experiments. Samples were dissolved in a medium salt buffer containing 2.5 mM Na_2HPO_4, 5 mM NaH_2PO_4, 100 mM NaCl, and 0.1 mM EDTA at pH = 7.0 with 1.0 μM concentrations of the two complementary oligonucleotide sequences. The increase in UV absorbance at 260 nm as a function of time was recorded while the temperature was increased linearly from 10 to 75 °C at a rate of 1.0 °C/min by means of a Peltier temperature programmer. The melting curves were found to be reversible.

CD Spectroscopy. CD spectra (200−350 nm) were recorded on a CD spectrometer as an average of five scans using a split of 2.0 nm and a scan speed of 50 nm/min. Samples were dissolved in a medium salt buffer containing 2.5 mM Na_2HPO_4, 5 mM NaH_2PO_4, 100 mM NaCl, and 0.1 mM EDTA at pH = 7.0 with 2.0 or 1.0 μM concentrations of the two complementary oligonucleotide sequences, heated to 80 °C and cooled to 10 °C. Quartz optical cells with a path length of 5.0 mm/10.0 mm were used.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

MALDI-TOF data for oligonucleotides. UV melting curves and CD spectra. Further T_m data of duplexes formed between the various modified sequences shown in Table 1. Selected NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Endo, M.; Sugiyama, H. *ChemBioChem* **2009**, *10*, 2420−2443.
(2) Lu, C.-H.; Willner, B.; Willner, I. *ACS Nano* **2013**, *7*, 8320−8332.
(3) Simmel, F. C. *Chem. Unserer Zeit* **2013**, *47*, 164−173.
(4) Malinovskii, V. L.; Wenger, D.; Häner, R. *Chem. Soc. Rev.* **2010**, *39*, 410−422.
(5) Bandy, T. J.; Brewer, A.; Burns, J. R.; Marth, G.; Nguyen, T.; Stulz, E. *Chem. Soc. Rev.* **2011**, *40*, 138−148.
(6) Deleavey, G. F.; Damha, M. J. *Chem. Biol.* **2012**, 937−954.
(7) Switzer, C.; Prakash, T. P.; Ahn, Y. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 815−818.
(8) Wu, T.; Froeyen, M.; Schepers, G.; Mullens, K.; Rozenski, J.; Busson, R.; Van Aershot, A.; Herdewijn, P. *Org. Lett.* **2004**, *6*, 51−54.
(9) Pedersen, S. L.; Nielsen, P. *Org. Biomol. Chem.* **2005**, *3*, 3570−3575.
(10) Wu, T.; Nauwelaerts, K.; Van Aershot, A.; Froeyen, M.; Lescrinier, E.; Herdewijn, P. *J. Org. Chem.* **2006**, *71*, 5423−5431.
(11) Christensen, M. S.; Madsen, C. M.; Nielsen, P. *Org. Biomol. Chem.* **2007**, *5*, 1586−1594.
(12) Andersen, C.; Sharma, P. K.; Christensen, M. S.; Steffansen, S. I.; Madsen, C. M.; Nielsen, P. *Org. Biomol. Chem.* **2008**, *6*, 3983−3988.
(13) Christensen, M. S.; Bond, A.; Nielsen, P. *Org. Biomol. Chem.* **2008**, *6*, 81−91.
(14) Umemoto, T.; Wengel, J.; Madsen, A. S. *Org. Biomol. Chem.* **2009**, *7*, 1793−1797.
(15) Shaikh, K. I.; Madsen, C. S.; Nielsen, L. J.; Jørgensen, A. S.; Nielsen, H.; Petersen, M.; Nielsen, P. *Chem.—Eur. J.* **2010**, *16*, 12904−12919.
(16) Kielkowski, P.; Pohl, R.; Hocek, M. *J. Org. Chem.* **2011**, *76*, 3457−3462.
(17) Jørgensen, A. S.; Shaikh, K. I.; Enderlin, G.; Ivarsen, E.; Kumar, S.; Nielsen, P. *Org. Biomol. Chem.* **2011**, *9*, 1381−1388.
(18) Madsen, C. S.; Witzke, S.; Kumar, P.; Negi, K.; Sharma, P. K.; Petersen, M.; Nielsen, P. *Chem.—Eur. J.* **2012**, *18*, 7434−7442.
(19) Madsen, C. S.; Nielsen, L. J.; Pedersen, N. S.; Lauritsen, A.; Nielsen, P. *RSC Adv.* **2013**, *3*, 10696−10706.
(20) Kumar, P.; Sharma, P. K.; Madsen, C. S.; Petersen, M.; Nielsen, P. *ChemBioChem* **2013**, *14*, 1072−1074.
(21) Kumar, P.; Madsen, C. S.; Nielsen, P. *Bioorg. Chem. Med. Lett.* **2013**, *23*, 6847−6850.
(22) Kumar, S.; Steffansen, S. I.; Albæk, N.; Nielsen, P. *Tetrahedron* **2014**, *70*, 583−589.
(23) Kumar, P.; Sorinas, A. F.; Nielsen, L. J.; Slot, M.; Skytte, K.; Nielsen, A. S.; Jensen, M. D.; Sharma, P. K.; Vester, B.; Petersen, M.; Nielsen, P. *J. Org. Chem.* **2014**, *79*, 8020−8030.
(24) Lemaire, S.; Houpis, I.; Wechselberger, R.; Langens, J.; Vermeulen, W. A. A.; Smets, N.; Nettekoven, U.; Wang, Y.; Xiao, T.; Qu, H.; Liu, R.; Jonckers, T. H. M.; Raboisson, P.; Vandyck, K.; Nilsson, K. M.; Spiro, V. F. *J. Org. Chem.* **2011**, *76*, 297−300.
(25) Sano, T.; Shuto, S.; Inoue, H.; Ueda, T. *Chem. Pharm. Bull.* **1985**, *33*, 3517−3662.
(26) T_m values for duplexes containing $\mathbf{U}_\mathbf{T}$ or $\mathbf{U}_\mathbf{A}$ have been remeasured for direct comparison and are 1−2 °C lower than published values,[18,20] following the use of new UV/Peltier equipment.