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Analogues of a Locked Nucleic Acid with Three-Carbon 2′,4′-Linkages: Synthesis by Ring-Closing Metathesis and Influence on Nucleic Acid Duplex Stability and Structure

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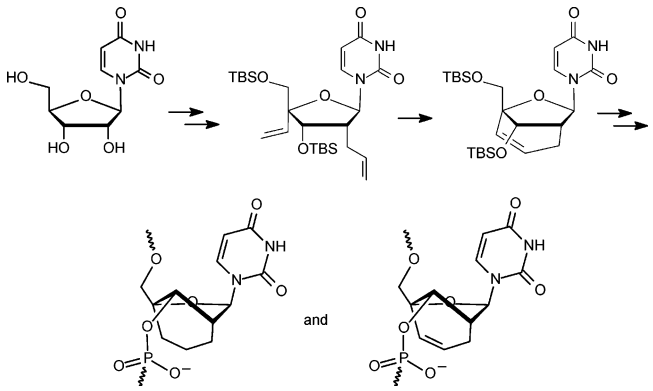

Two bicyclic 2′-deoxynucleoside analogues containing a saturated and an unsaturated three-carbon 2′,4′-linkage, respectively, have been synthesized using a ring-closing metathesis-based linear strategy starting from uridine. Both analogues have been incorporated into oligodeoxynucleotide sequences and increased the stability of DNA:RNA hybrid duplexes ($\Delta T_m \sim 2.5-5.0$ °C per modification) and decreased the stability of dsDNA duplexes ($\Delta T_m \sim 2.5-1.0$ °C per modification). CD spectroscopy revealed that the bicyclic nucleosides induced formation of A-type-like duplexes albeit to a lesser degree than found for locked nucleic acid (LNA) monomers. From the CD data and UV melting analysis, we propose that the 2′-oxygen atom of the bicyclic moiety is essential for the formation of stabilized A-type-like dsDNA but not for the formation of a stabilized A-type DNA:RNA hybrid.

Introduction

The molecular recognition between two complementary nucleic acid sequences forming a duplex structure is a key subject for investigations in chemical biology and biomimetic chemistry.[1] The search for small chemical perturbations leading to an increased thermal stability of a duplex without compromising the nature and selectivity of the Watson−Crick base pairing has been very successful by the introduction of conformationally restricted nucleoside building blocks.[2] The most dramatic increases in stability have been obtained with a strong restriction of the carbohydrate moieties into the N-type (or C-3′-*endo*) conformations (Figure 1) leading to the formation of A-type or A-type-like duplexes.[3−7] The prime example is LNA (locked nucleic acid), defined as a nucleic acid containing one or several replacements of the natural nucleosides with the bicyclic nucleosides **1**.[3] In **1**, a 2′,4′-connection locks the sugar

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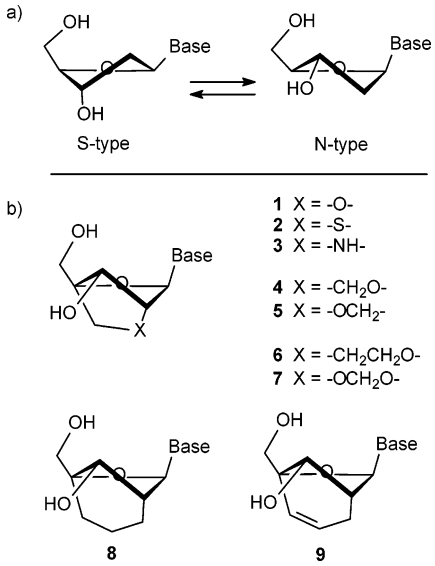

FIGURE 1. (a) Low-energy conformations of 2′-deoxynucleosides. (b) Bicyclic nucleosides with 2′,4′-linkages and locked N-type conformations.

conformation of the building block into a perfect N-type conformation (Figure 1).[3] Unprecedented stability of duplexes has been observed with short LNA oligonucleotides containing entirely LNA nucleosides or mixmers of both LNA and unmodified ribo- or 2′-deoxyribonucleosides with complementary sequences being RNA, DNA, or other LNA sequences.[3] The increase in thermal stability compared to unmodified DNA duplexes ranges from +3 to +8 °C for each incorporation of an LNA nucleoside.[3] Owing to this high potential in the recognition of complementary natural DNA and RNA sequences, LNA sequences are now in use as diagnostic materials and under study as therapeutic compounds following the antisense approach[8] or as LNAzymes.[9] The intriguing properties of LNA have inspired structural research by NMR[10,11] and X-ray crystallography,[12] and it was shown that the LNA nucleosides drive their neighboring 2′-deoxynucleosides into N-type conformations leading to overall A-type or A-type-like duplexes even with only three LNA nucleosides in a nonamer duplex.[11]

The features of LNA have led to a number of closely related analogues including stereoisomers of LNA[13,14] and analogues in which the 2′,4′-bridge has been altered (Figure 1). Both the sulfur analogue, **2**, and the amino analogue, **3**, give virtually the same increases in thermal stability as the parent oxo-compound **1** (ΔT_m's for each modification in an oligodeoxynucleotide (ODN) range between +3 and +5 °C with DNA complements and between +4 and +8 °C with RNA complements).[15] The 2′-nitrogen of amino-LNA **3** has been used for attaching other chemical groups into the stabilized A-type duplex.[16] Nucleosides with three atoms in the 2′,4′-linkage have also been reported, and **4**, named ENA, displays a stabilization of nucleic acid duplexes formed with complementary RNA that is equal or slightly lower than that found for LNA (ΔT_m's between +3.5 and +5.5 °C in a mixed-sequence context).[17,18] The closely related analogue **5** where the additional methylene group was inserted between the 2′-carbon and the oxygen displayed somewhat smaller increases compared to LNA (ΔT_m's between +2 and +3 °C with RNA complements).[19] With complementary DNA, **4** shows only a small stabilization (ΔT_m's between +0.5 and +2 °C)[17,18] whereas **5** shows almost unchanged melting temperatures compared to a native DNA duplex (ΔT_m's between −0.5 and +1 °C).[19] Two nucleosides with a four-atom 2′,4′-linkage have also been synthesized, and in the case of **6**, a destabilization of duplexes with both DNA and RNA complements was seen (one measured ΔT_m of −2.0 °C with a DNA and of −0.5 °C with an RNA complement, respectively).[18] Exchanging one carbon atom with oxygen led to **7**, which recently showed a moderate stabilization of a duplex with complementary RNA (ΔT_m's between +1 and +2 °C) and

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a corresponding destabilization with complementary DNA (ΔT_m's between −1 and −2 °C).[20]

The interesting carbocyclic analogue of LNA/ENA, **8** (base = T), was mentioned briefly in a review almost a decade ago.[21] Therein, no synthetic details and only one melting temperature were given claiming a decrease in thermal stability of −2.3 °C for one central incorporation of **8** in a 17-mer ODN against a complementary RNA sequence.[21] In terms of the results obtained with other LNA analogues, especially **4** and **5** with an equal length of the 2′,4′-linkage, this result seems somewhat surprising, and we decided to synthesize **8** to explore whether a direct correlation between the constitution of the 2′,4′-linkage in the bicyclic nucleoside, including the presence and position of any oxygen atom, and the influence in thermal stability of duplexes can be revealed. We here present the synthesis of **8** using a ring-closing metathesis-based strategy. Besides **8**, this strategy gives access to the equally interesting unsaturated bicyclic nucleoside **9**. Both compounds, analyzed with uracil as the nucleobase, enlighten the scope for conformational restriction and present additional data on the importance of the 2′-oxygen atom in nucleic acid duplexes.

Results

Chemical Synthesis. As mentioned above, we planned to introduce the additional 2′,4′-bridge into the desired nucleosides via a ring-closing metathesis (RCM) reaction. This type of reaction has been successfully used on nucleoside and nucleotide substrates by us[22,23] and others[24] for making varying sizes of rings, including a bicyclic nucleoside with a conformationally restricting 3′,4′-linkage.[25] The RCM transformation leading to our target nucleosides **8** and **9** requires a 2′-deoxynucleoside substrate with a vinyl group in the 4′-C position and an allyl group in the 2′-C position. Because efficient methods for introducing both of these two alkyl groups into nucleosides (though never in the same molecule) have been presented in the literature,[23,26−29] we decided to follow a linear synthetic strategy starting from uridine. Hence, this should be the shortest possible route, and we decided to start with the deoxygenative introduction of the 2′-*C*-allyl group. The protecting group most often used for the 3′- and 5′-hydroxy groups in ribonucleosides while introducing the 2′-*C*-allyl group[28] or a plethora of 2′-*O*-alkyl groups[5] is the elegantly selective bidentate TIPDS group developed by Markiewicz.[30] However, we decided to use the less commonly used selective protection of the same hydroxy groups with the *tert*-butyldimethylsilyl (TBS) group as reported by Hakimelahi et al.[31] This allows us to perform the same desired transformation in the 2′-position,[29] and more importantly, it subsequently allows a selective acidic deprotection of the 5′-position.[23] Thus, the 3′-*O*-TBS group can be maintained as a permanent protection during the entire synthesis. We expected that the 4′-*C*-vinyl group could be introduced on the 5′-O-unprotected nucleoside through a series of steps involving oxidation, aldol condensation to introduce a hydroxymethyl group,[3,19,32] selective protection,[3,23,27,32] oxidation, and a Wittig reaction.[18,23,27] The nucleoside would subsequently be set up to combine the allyl and the vinyl groups in an RCM reaction, leading after deprotection to **9** (base = U) and after hydrogenation to **8** (base = U).

The 3′,5′-di-*O*-TBS-protected uridine **10** (Scheme 1) was synthesized from uridine in one step using the literature method.[31] The method is robust leading to the product **10** in 90% isolated yield after a chromatographic purification to remove small amounts of the 2′,5′-protected isomer. Compound **10** was in two steps converted to the 2′-*C*-allyl derivative **12**[29] in 51% overall yield using a 2′-*O*-phenoxythiocarbonyl intermediate **11** and a radical reaction with allyltributyltin.[28,29] The removal of the 5′-*O*-TBS group was achieved most selectively in a slow treatment with 80% acetic acid at room temperature[23] over 3 days giving **13** in 75% yield. Attempts to shorten the reaction time by heating the reaction mixture or using other acids resulted in removal of both TBS groups. The primary hydroxy group in **13** was oxidized with Dess−Martin periodinane,[33] and the resulting aldehyde was reacted with formaldehyde in an aldol condensation. After reduction with sodium borohydride, the diol **14** was isolated in 52% overall yield from **13**. This method for introducing a 4′-hydroxymethyl group has been previously reported on a range of nucleoside substrates usually giving comparable yields.[32] A protection of the 5′-hydroxy group was necessary to allow the introduction of a 4′-*C*-vinyl group. However, in all similar molecules, it has been shown that the 4′-*C*-hydroxymethyl group is the more reactive of the two primary hydroxy groups.[23,27,32] Eventually, a three-step procedure was devised, where protection of the 4′-hydroxymethyl was followed by protection of the 5′-hydroxy group and finally by a removal of the 4′-hydroxymethyl protecting group. Different approaches were attempted using a DMT, a TBS, or a benzoyl group in the first protection step. In our hands, benzoylation followed by purification, TBS protection, and deprotection of the benzoyl group gave the best result. Obviously, a resulting nucleoside with TBS groups in both the 3′-O

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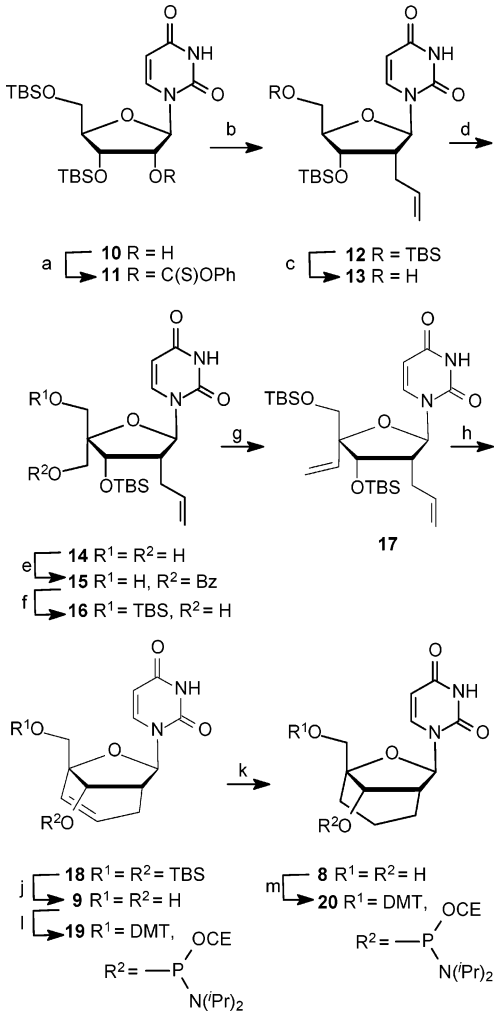
SCHEME 1. Synthesis of Bicyclic Nucleosides[a]



[a] Reagents and conditions: (a) PhOC(S)Cl, DMAP, CH_3CN (79%); (b) allyltributyltin, AIBN, toluene (65%); (c) 80% CH_3COOH (75%); (d) (i) Dess−Martin periodinane, CH_2Cl_2, (ii) HCHO, 2 M NaOH, dioxane, then $NaBH_4$ (52%); (e) BzCl, pyridine, CH_3CN (65%); (f) (i) TBSCl, imidazole, DMF, (ii) $NaOCH_3$, CH_3OH (71%); (g) (i) Dess−Martin periodinane, CH_2Cl_2 (93%), (ii) BuLi, Ph_3PBrCH_3, THF (95%); (h) Grubbs second-generation catalyst, CH_2Cl_2 (96%); (j) TBAF, THF (98%); (k) from **9**, H_2, PtO_2, CH_3OH (100%); (l) (i) DMTCl, pyridine, CH_3CN (78%), (ii) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, $EtN(^iPr)_2$, CH_2Cl_2 (57%); (m) (i) DMTCl, Et_3N, CH_2Cl_2 (78%), (ii) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, $EtN(^iPr)_2$, CH_2Cl_2 (63%). TBS = *tert*-butyldimethylsilyl. DMT = 4,4′-dimethoxytrityl. CE = 2-cyanoethyl.

and 5′-O positions gives the advantage of a combined removal of both on the final nucleoside. Initial attempts to perform regioselective benzoylation of the diol gave only one mono-protected isomer **15** as well as the dibenzoyl derivative. Therefore, a procedure using only 0.9 equiv of benzoyl chloride was chosen. No dibenzoyl isomer was formed, and **15** was isolated in 65% yield along with 28% recovered starting material **14**, i.e., a 90% effective yield of **15**. The TBS protection of the remaining hydroxy group using TBSCl and imidazole followed by removal of the benzoyl group with sodium methoxide gave **16** in 71% overall yield. Oxidation with Dess−Martin periodinane gave the aldehyde, which was reacted in a Wittig reaction, and the diene **17** was isolated in a very good 88% yield over the two steps. The two terminal alkenes in **17** were reacted in a ring-closing metathesis (RCM) reaction using Grubbs' second-generation catalyst, $((Mes)_2Im)(Cy_3P)Cl_2Ru$=CHPh,[34] in refluxing dichloromethane, and the resulting bicyclic nucleoside **18** was isolated in an excellent 96% yield. The fact that the ring closure was performed confirmed the 2′,4′-cis positioning of the olefins respective to the furanose ring and thereby the 4′-configuration of **15** and **16**. The silyl protecting groups of **18** were removed with TBAF to give the target nucleoside **9** in 98% yield. Hydrogenation of the double bond in **9** was performed in methanol using Adams' catalyst, and the saturated target bicyclic nucleoside **8** was isolated in quantitative yield. The structures of the target nucleosides **8** and **9** were confirmed by NMR and mass spectrometry, and a more thorough conformational study was performed (see below). To prepare nucleosides **8** and **9** for automated oligonucleotide synthesis, both were in two steps converted to the appropriately protected phosphoramidites. Thus, the primary 5′-hydroxy groups were protected with the DMT group by standard protocols, and afterward, the 3′-*O*-phosphoramidites **19** and **20** were obtained in acceptable yields.

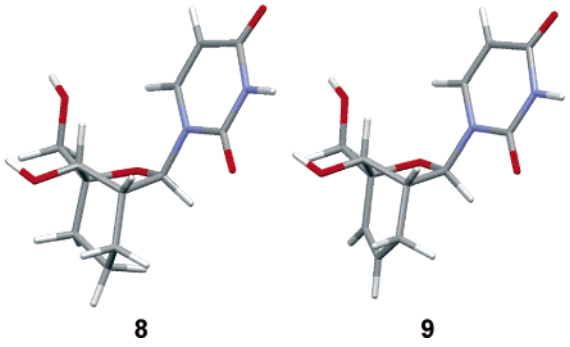


FIGURE 2. Optimized geometry of **8** and **9** obtained by ab initio calculations.

Conformational Analysis. The ^1H NMR spectra of **8** and **9** show the three-bond coupling constant $^3J_{H1'H2'} = 0$ as found for all bicyclic nucleosides with 2′,4′-linkages. This clearly indicates a locked N-type conformation. On the other hand, no significant structural information about the carbocyclic ring can be taken from the ^1H NMR spectrum of **8** because of a severe overlap of signals. Ab initio calculations revealed the lowest-energy conformations of the bicyclic nucleosides **8** and **9**, as shown in Figure 2. Both nucleosides are locked in N-type conformations but with slightly different sugar puckering (Table 1). The cyclohexane ring of **8** adopts a perfect chair conformation, and the cyclohexene ring of **9** is in a near-envelope conformation with five atoms in a plane. Table 1 compares the pseudorotation angles, *P*, and puckering amplitudes[35] for various bicyclic nucleosides with a locked N-type conformation. X-ray data were used when available, i.e., for **1**, **4**, and **7**, whereas ab initio calculations were performed on **5** and **6** in the same way as those with **8** and **9**. Ab initio calculations of **1** and **4** give results (data not shown) in accordance with X-ray data, validating the calculations. All the monomers are locked in N-type conformations with *P* in the range from 11° to 21°, except for **9** with $P = 27°$. The puckering amplitude strictly follows the size of the additional ring: for two-atom 2′,4′-linkages, ν_{max} is around 57°; for three-atom 2′,4′-linkages, ν_{max}

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(35) For definitions and nomenclature regarding nucleoside and nucleic acid structure and conformations, see: Saenger, W. *Principles of Nucleic Acid Structure*; Springer: New York, 1984.

TABLE 1. Sugar Puckering Data for Different Bicyclic Nucleosides with 2′,4′-Linkages[a]

	2′,4′-linkage	pseudorotation angle *P*	puckering amplitude ν_{max}
1	4′-CH_2O-2′	17° [b]	57° [b]
		15° [b]	57° [b]
		20° [c]	58° [c]
4	4′-CH_2CH_2O-2′	15° [c]	48° [c]
		12° [d]	48° [d]
5	4′-CH_2OCH_2-2′	20° [e]	48° [e]
8	4′-$CH_2CH_2CH_2$-2′	15° [e]	46° [e]
9	4′-CH═$CHCH_2$-2′	27° [e]	49° [e]
6	4′-$CH_2CH_2CH_2O$-2′	19° [f]	38° [f]
7	4′-CH_2OCH_2O-2′	17° [g]	38° [g]
	A-type RNA	14° [h]	38° [h]

[a] Data obtained from literature X-ray data or ab initio calculated energy-minimized structures. [b] Two different conformations are in the unit cell: X-ray data, base = T, ref 18. [c] X-ray data, base = A, ref 18. [d] X-ray data, base = isobutyl-G, ref 18. [e] Ab initio data from the present study, base = U. [f] Ab initio data from the present study, base = T. [g] X-ray data, base = T, ref 20. [h] Taken from ref 35.

TABLE 2. Thermal Stability Data of Modified Duplexes

	ODN sequences[a]	complementary DNA 5′-dGCATATCAC-3′ T_m (ΔT_m)/°C[b]	complementary RNA 5′-rGCAUAUCAC-3′ T_m (ΔT_m)/°C[b]
21	5′-dGTGATATGC-3′	30.0	27.5
22	5′-dGTGA**X**ATGC-3′	29.0 (−1.0)	32.0 (+4.5)
23	5′-dG**X**GA**X**A**X**GC-3′	27.0 (−1.0)	38.0 (+3.5)
24	5′-dGTGA**Y**ATGC-3′	29.0 (−1.0)	31.5 (+4.0)
25	5′-dG**Y**GA**Y**A**Y**GC-3′	23.0 (−2.3)	34.5 (+2.3)

[a] Oligodeoxynucleotide sequences with **X** = **8** (incorporation of **20**) and **Y** = **9** (incorporation of **19**). [b] Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in a buffer containing 5 mM Na_2HPO_4, 10 mM NaH_2PO_4, 100 mM NaCl, and 0.1 mM EDTA, pH 7.0, using 1.5 μM concentrations of each strand. Values in brackets show the changes in T_m values per modification compared with the reference strand **21**.

is around 48°; and for four-atom 2′,4′-linkages, ν_{max} is around 38° (the value for ribose/deoxyribose).

Synthesis and Hybridization Properties of Oligonucleotides. The two phosphoramidites **19** and **20** were used as building blocks in the synthesis of oligonucleotides using a standard automated solid-phase method. Both phosphoramidites **19** and **20** were coupled in high yields and incorporated into 9-mer ODN sequences with either one or three modifications (Table 2). For the study, we have chosen the same 9-mer sequence as that employed in the first study of LNA and other nucleic acid analogues which will ease comparison.[3,15] The composition of the modified oligonucleotides was verified by MALDI MS. The hybridization of the modified oligonucleotides toward complementary DNA and RNA sequences was studied by thermal denaturation experiments. The melting temperatures of the formed duplexes were determined and compared with the unmodified duplexes (Table 2). These experiments show that a single incorporation of the bicyclic nucleoside **8** in an ODN increases the stability of the duplex formed with RNA with 4.5 °C. In the case of three modifications, a stabilization of 3.5 °C per modification is seen. The similar incorporations of the other bicyclic nucleoside containing an unsaturated 2′,4′-linkage **9** lead to slightly smaller increases in thermal stability of 4.0 and 2.3 °C per modification, for one and three modifications, respectively. Hybridization of the same modified sequences with a DNA complement demonstrates a general

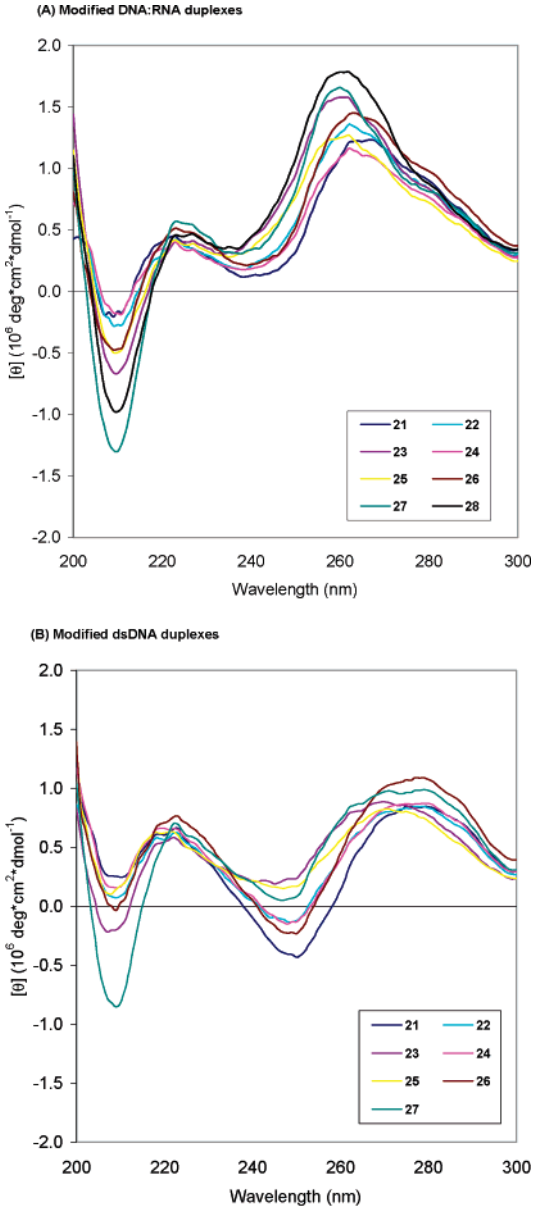

FIGURE 3. CD spectra of duplexes formed by the oligonucleotides **21**−**28** and their DNA and RNA complements. (A) Modified DNA:RNA duplexes. (B) Modified dsDNA duplexes. Sequences **26** and **27** correspond to the LNA sequences 5′-dGTGA**T**$^{\mathbf{L}}$ATGC-3′ and 5′-dG**T**$^{\mathbf{L}}$GA**T**$^{\mathbf{L}}$A**T**$^{\mathbf{L}}$GC-3′, respectively, where **T**$^{\mathbf{L}}$ is the LNA thymidine monomer **1**. Sequence **28** corresponds to the RNA sequence 5′-rGUGAUAUGC-3′.

destabilization in the range of −1 °C for each of the incorporations, even though a more pronounced destabilization of −2.3 °C for three incorporations of **9** was observed.

Circular Dichroism Spectroscopy. CD spectra were recorded for all duplexes of the study (Table 2) as well as for the corresponding duplexes with one or three incorporations of LNA **1** (base = T) and a dsRNA duplex (Figure 3A). A- and B-type duplex geometries have distinctly different CD features. A-type duplexes give an intense negative band at ∼210 nm and a positive one at ∼260 nm of approximately equal magnitude as exemplified by the dsRNA in Figure 3. B-type duplexes give much less of a CD signal than A-type duplexes, as there is no negative signal at ∼210 nm and negative and positive bands of relatively low intensity at ∼250 nm and ∼275 nm, respec-

tively.[36] The DNA:RNA duplex with three LNA modifications clearly displays the A-type characteristics found for the RNA:RNA duplex (compare **27** and **28**, Figure 3A). Thus, the negative band at 210 nm is intense for both duplexes and small for the unmodified DNA:RNA duplex (**21**). For the duplexes modified with **8** or **9**, this band is of lower intensity than that observed with LNA and clearly decreases in intensity in the order LNA > **8** > **9** > DNA for both one (i.e., **26**, **22**, and **24**) and three incorporations (i.e., **27**, **23**, and **25**) in the DNA:RNA duplex. A similar picture can be seen with the positive band around 260 nm. Thus, three LNA modifications result in the complete shift of a DNA:RNA duplex toward an A-type duplex, whereas this effect is significantly smaller with three incorporations of **8** and even smaller with **9**.

The unmodified dsDNA duplex is a pure B-type duplex, whereas all the modified dsDNA duplexes seem to be changed toward an intermediate duplex form (Figure 3B). The deviation from the standard B-type geometry is clearly followed in the decreasing bands at 210 and 250 nm, and it follows the same trend as that seen for the DNA:RNA duplexes. Thus, the duplexes containing **9** (i.e., **24** and **25**) are less altered than the duplexes containing **8** (i.e., **22** and **23**) which on the other hand are considerably less altered than the LNA-containing duplexes (i.e., **26** and **27**). As expected, three modifications lead to larger deviations than one modification for each of the three different bicyclic nucleic acid analogues.

Discussion

The synthesis of **8** and **9** was easily accomplished using the linear strategy from uridine. Thus, the ring-closing key step by an RCM reaction was extremely efficient in producing the conformationally restricting cyclohexene ring. The drawback of the linear strategy is, of course, that the access to the analogues of **8** and **9** with other nucleobases relies on repeating the complete series of steps starting from the other natural ribonucleosides. However, the cytosine analogues should be readily accessible from **18** through a standard U to C conversion. The olefin of **9** opens the opportunity of preparing a range of analogues of **8** that are functionalized on the carbocyclic ring.

The observed increase in RNA affinity obtained with **8** (base = U) is significant and surprising with regards to the brief report on **8** (base = T) given in the literature[21] which claims decreased RNA affinity. The discrepancy might be explained by different sequence contexts including different nucleobases of the bicyclic nucleoside, although this seems unlikely. The fact that the nucleosides **8** and **9** presented in our study are uridine and not thymidine analogues should be taken into account but points in the opposite direction. Thus, even higher melting temperatures should be expected for the corresponding thymidine analogues due to the well-known positive influence of the 5-methyl group (~1 °C per methyl group).

With the present data for the two new members of the series of bicyclic nucleosides with 2′,4′-linkages and locked N-type conformations (Figure 1), the overall relation between duplex structure and stability vs the nature of the bicyclic building blocks can be further enlightened. When comparing the sugar puckering data (Table 1), it is clear that the length of the 2′,4′-linkage directly determines the puckering amplitude of the bicyclic nucleoside, whereas the constitution of the 2′,4′-linkage is of no influence. Little variation in the pseudorotation angle (11°−27°) is observed describing near ^{3}E envelope conformations in all cases. In the case of an ^{3}E envelope sugar conformation, the puckering amplitude describes the deviation of the C-3′ atom from the plane of the remaining sugar atoms. Changes in the puckering amplitude and the position of C-3′ lead to changes in the δ backbone angle, which has a value of ~65°, ~75°, and ~80° for the two-, three-, and four-atom 2′,4′-linkages, respectively (data not shown). At first glance, the stabilization of duplexes follows the length of the 2′,4′-linkage and thereby the puckering amplitude or value of δ. We note, however, that the difference in duplex stabilization between LNA (**1**, two-atom linkage) and ENA (**4**, three-atom linkage) is small if existent at all.

In a direct comparison of the influence of different three-atom linkages, **8** shows an influence on duplex stability comparable to what has been reported for its oxa-analogues **4** and **5**. Hence, in comparable though not identical sequences, **8** leads to an increase in the stability of a DNA:RNA hybrid similar to that seen for **4**[17,18] (i.e., ΔT_m's between +3.5 and +5.5 °C per modification). In comparison, **5**[19] as well as our unsaturated analogue **9** lead to slightly smaller increases in stability (ΔT_m's between +2 and +4 °C per modification). It should, of course, be taken into account that the sequence contexts are different. The sequence used in the present study of **8** and **9** is the same 9-mer sequence as that used in our original study of LNA **1** and later in the study of sulfur and amino analogues **2** and **3**. The melting temperatures were in all cases somewhat higher for **1**, **2**, or **3** (45−50 °C) than for **8** (38 °C, **23**, Table 2). The same sequence has not been studied with ENA **4**. In the original study of ENA, however, a direct comparison with LNA in equal oligopyrimidine sequences revealed almost identical melting temperatures.[17] In the context of a dsDNA duplex, the modifications with a three-atom 2′,4′-linkage range from a considerable stabilization reported for **4**,[17,18] and a neutral influence reported for **5**,[19] to slight destabilizations seen for both **8** and **9** (Table 2). Again, a direct comparison of ENA and LNA (**4** and **1**) revealed similar increases in T_m.[17] The general observation with the bicyclic nucleosides with three-atom 2′,4′-linkages is, therefore, that the variation is large ranging from the unprecedented duplex stabilization similar to that seen with two-atom 2′,4′-linkages (LNA and analogues, **1**−**3**) to the more or less neutral stability similar to that generally seen with the four-atom 2′,4′-linkages (**6** and **7**). From the data in hand, it therefore appears that the atomic constitution of the 2′,4′-linkage is more important than its length. When comparing the different three-atom linkers, the exact position of the oxygen emerges as a very important feature for duplex stabilization, and more so in dsDNA duplexes than in DNA:RNA hybrid duplexes.

It has been suggested that a major feature responsible for the ability of LNA−DNA mixmers to form stabilized A-type duplexes with complementary RNA (and A-type-like duplexes with complementary DNA) is the conformational steering of neighboring 2′-deoxynucleotides toward N-type sugar puckers.[11] As indicated from the CD spectra (Figure 3), this effect is less pronounced when the LNA monomers are replaced with **8** and even further reduced by replacement with **9**. Thus, the conformational steering is, apparently, reduced by the hydrophobic nature of the 2′,4′-linkage in **8** and **9**. It is noteworthy that ENA and LNA increase the thermal stability of duplexes equally, and from CD spectra, it also appears that they perturb the duplex

(36) Rodger, A.; Nordén, B. *Circular dichroism and linear dichroism*; Oxford University Press: Oxford, 1997.

structure comparably.[18] Overall, for LNA, ENA, **8**, and **9**, there is a strong correlation between duplex structure and stability, i.e., the more conformational steering (and consequently A-type characteristics of a given duplex), the higher the thermal stability. It is interesting that the degree of conformational steering depends on the presence of a 2′-oxygen atom. Considering specific duplex types, a larger difference in melting temperature is observed for dsDNA duplexes than for DNA:RNA duplexes going from LNA and ENA to **8** and **9**. In the case of DNA:RNA duplexes, the locked N-type conformation leads to a general gain in thermal stability, whereas the presence of a 2′-oxygen is crucial for optimizing the effect of the locked N-type conformation in dsDNA. In other words, the 2′-oxygen is more important for conformational steering and duplex stability in the case of dsDNA than for DNA:RNA.

A dsDNA duplex has a B-type geometry with a narrow minor groove which is extensively hydrated (spine of hydration),[37] whereas a DNA:RNA duplex possesses a geometry intermediate between A- and B-type, albeit closer to A- than B-type, with a fairly wide minor groove. As a result, the change in duplex geometry upon inclusion of N-type nucleotides is larger for a dsDNA duplex (B-type to intermediate geometry) than for a DNA:RNA duplex (intermediate to A-type geometry). It is possible that the 2′-oxygen is important for maintaining a water network upon the widening of the minor groove in going from a B-type to an intermediate duplex geometry. In this case, it would imply that the cooperative conformational steering of neighboring nucleotides into N-type sugar puckers is driven by the hydration of the minor groove. The hybridization properties of oligonucleotides with **8** and **9** incorporated toward RNA complements show that the hydrophobic 2′,4′-linkers are equally as well tolerated as the hydrophilic linkers of LNA and ENA in this context. This could imply that minor groove hydration is less important for A-type duplexes.

The difference in duplex stabilization between **8** and **9** is difficult to explain. The CD spectra show that incorporation of **8** leads to a larger change in global duplex structure toward A-type than the incorporation of **9**. This could possibly be due to differential influence on the minor groove hydration by **8** and **9**, but we also note that the sugar pucker of **9** ($P = 27°$) is slightly different compared to the other nucleic acid analogues discussed in this paper (Table 1).

In summary, the RNA-selective recognition of oligonucleotides containing **8** might be a useful property. Thus, the RNA affinity is almost retained compared to LNA and ENA, whereas the DNA affinity is reduced below the level of unmodified ODNs. This difference in RNA affinity over DNA affinity is larger than that for any of the other bicyclic nucleic acid analogues with 2′,4′-linkages. If the nuclease-mediated degradation of oligonucleotides with **8** or **9** incorporated, as it might be expected, is reduced to the same extent as that reported for ENA,[18] **8** and **9** could be promising candidates in the development of antisense oligonucleotides. The relatively short and efficient synthetic route is a significant advantage in this perspective.

Conclusion

Two different modified nucleosides with a three-carbon 2′,4′-linkage were synthesized in the same good overall yield of 13% from uridine, using a linear strategy with a very efficient RCM reaction as the key step. Both were easily incorporated into ODNs and were found to stabilize DNA:RNA duplexes significantly and to destabilize dsDNA duplexes slightly. Comparison with other 2′,4′-linked nucleoside analogues leads to the conclusion that the position of a hydrophilic oxygen atom is more important in an A-type-like dsDNA duplex than in an A-type DNA:RNA duplex. It also indicates that the duplex enhancing property of this class of bicyclic nucleic acid building blocks in general, and of LNA in particular, might be related to water binding in the minor groove. Dependent on their pharmacokinetic properties, both the saturated carbocyclic LNA analogue **8** and its unsaturated equivalent **9** could be promising candidates in antisense research. The RNA-selective recognition mode can also be an advantage in diagnostic applications. The unsaturated moiety of **9** gives the opportunity for an easy synthetic access to a plethora of functionalized derivatives of **8** and **9**.

Experimental Section

Synthesis of 2′-Deoxy-2′-*C*-allyl-3′-*O*-(*tert*-butyldimethylsilyl)-uridine (13). A solution of **12** (0.766 g, 1.54 mmol) in 80% CH_3-COOH (20 mL) was stirred for 3 days. After neutralization with a saturated aqueous solution of $NaHCO_3$, the solution was extracted with CH_2Cl_2 (3 × 120 mL). The combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (80 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc−petroleum ether 2:3 v/v) to give the product **13** (0.450 g, 75%) as a white solid: R_f 0.3 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1H, NH), 7.56 (d, 1H, J = 8.0 Hz, H-6), 5.81 (d, 1H, J = 8.6 Hz, H-1′), 5.77−5.65 (m, 2H, H-5, C*H*═CH_2), 5.06 (dd, 1H, J = 1.4 Hz, J = 17.3 Hz, CH═C*H*$_2$), 4.99 (d, 1H, J = 10.1 Hz, CH═C*H*$_2$), 4.39 (dd, 1H, J = 1.7 Hz, J = 5.6 Hz, H-3′), 4.00 (m, 1H, H-4′), 3.88 (dt, 1H, J = 3.2 Hz, J = 11.9 Hz, H-5′), 3.76 (ddd, 1H, J = 2.8 Hz, J = 6.0 Hz, J = 11.9 Hz, H-5′), 2.83 (br s, 1H, OH), 2.56 (m, 1H, H-2′), 2.43 (m, 1H, C*H*$_2$CH═CH_2), 2.14 (m, 1H, C*H*$_2$CH═CH_2), 0.92 (s, 9H, SiC(CH_3)$_3$), 0.09 (s, 6H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.2 (C-4), 150.4 (C-2), 141.9 (C-6), 135.2 (*C*HCH$_2$), 116.8 (CH═*C*H$_2$), 102.6 (C-5), 91.6 (C-1′), 87.6 (C-4′), 73.8 (C-3′), 62.7 (C-5′), 47.6 (C-2′), 29.2 (*C*H$_2$-CH═CH_2), 25.7 (C(*C*H$_3$)$_3$), 18.0 (*C*(CH$_3$)$_3$), −4.5 (CH_3Si), −4.9 (CH_3Si); MALDI MS *m*/*z* (405.2 [M + Na]$^+$, $C_{18}H_{30}O_5N_2Si$ − Na^+ calcd 405.2).

Synthesis of 2′-Deoxy-2′-*C*-allyl-4′-*C*-hydroxymethyl-3′-*O*-(*tert*-butyldimethylsilyl)uridine (14). To a stirred solution of **13** (3.88 g, 10.1 mmol) in anhydrous CH_2Cl_2 (150 mL) was added Dess−Martin periodinane (4.45 g, 10.7 mmol). After 2 h, the reaction mixture was filtered through Celite and the sinter was rinsed with EtOAc (100 mL). The combined organic phases were washed with a mixture of a saturated aqueous solution of $NaHCO_3$ and a saturated aqueous solution of $Na_2S_2O_3$ (1:1, 120 mL), and the water phase was extracted with EtOAc (160 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure to give the aldehyde as a white foam. A solution of the foam in dioxane (100 mL) was added to HCHO (37%, 2.2 mL, 25 mmol) and a 2 M, aqueous solution of NaOH (6.5 mL, 13 mmol). After stirring for 17 h, the mixture was cooled to 0 °C and $NaBH_4$ (1.071 g, 28.3 mmol) was added. The reaction mixture was stirred at room temperature for 5 h, and then we added a mixture of CH_3COOH and pyridine (1:4, 10 mL) and H_2O (100 mL). The resulting mixture was extracted with $CHCl_3$ (3 × 150 mL), and the combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (50 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc) to give **14** (2.168 g, 52%) as a white solid: R_f 0.2 (EtOAc)

(37) Egli, M.; Tereshko, V.; Teplova, M.; Minasov, G.; Joachimiak, A.; Sanishvili, R.; Weeks, C. M.; Miller, M. R.; Maier, M. A.; An, H.; Cook, P. D.; Manoharan, M. *Biopolymers* **2000**, *48*, 234−252.

(Found: C, 54.69; H, 8.18; N, 6.56. $C_{19}H_{32}O_6N_2Si\cdot 1/2H_2O$ requires C, 54.13; H, 7.89; N, 6.65%); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17 (s, 1H, NH), 7.31 (d, 1H, J = 8.3 Hz, H-6), 5.75 (d, 1H, J = 8.8 Hz, H-1′), 5.72 (d, 1H, J = 8.3 Hz, H-5), 5.58 (m, 1H, C*H*=CH$_2$), 5.06 (dd, 1H, J = 0.9 Hz, J = 17.1 Hz, CH=C*H*$_2$), 4.98 (d, 1H, J = 10.1 Hz, CH=C*H*$_2$), 4.60 (d, 1H, J = 6.6 Hz, H-3′), 3.86 (dd, 1H, J = 3.5 Hz, J = 12.2 Hz, CH$_2$O), 3.78 (d, 1H, J = 3.9 Hz, J = 11.5 Hz, CH$_2$O), 3.67 (dd, 1H, J = 7.0 Hz, J = 11.5 Hz, CH$_2$O), 3.57 (dd, 1H, J = 9.8 Hz, J = 12.2 Hz, CH$_2$O), 2.83−2.76 (m, 2H, OH, H-2′), 2.38 (m, 1H, C*H*$_2$CH=CH$_2$), 2.30−2.16 (m, 2H, C*H*$_2$CH=CH$_2$, OH), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.13 (s, 6H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.3 (C-4), 150.5 (C-2), 141.9 (C-6), 135.0 (*C*H=CH$_2$), 117.1 (CH=*C*H$_2$), 102.7 (C-5), 91.3, 88.8 (C-1′, C-4′), 74.8 (C-3′), 64.2, 63.8 (C-5′, CH$_2$O), 47.2 (C-2′), 30.4 (*C*H$_2$CH=CH$_2$), 25.9 (C(*C*H$_3$)$_3$), 18.1 (*C*(CH$_3$)$_3$), −4.4 (CH$_3$Si), −4.6 (CH$_3$Si); HRMALDI MS *m*/*z* (435.1922 [M + Na]$^+$, $C_{19}H_{32}O_6N_2Si$ − Na$^+$ calcd 435.1923).

Synthesis of 2′-Deoxy-2′-*C*-allyl-4′-*C*-benzoyloxymethyl-3′-*O*-(*tert*-butyldimethylsilyl)uridine (15). A stirred solution of **14** (2.151 g, 5.21 mmol) in anhydrous CH_3CN (25 mL) and anhydrous pyridine (25 mL) was cooled to 0 °C, and BzCl (0.50 mL, 4.3 mmol) was added. The reaction mixture was stirred at room temperature, and after 1.75 h H_2O (50 mL) was added. The resulting mixture was extracted with $CHCl_3$ (3 × 100 mL), and the combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (50 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc−petroleum ether 2:3 v/v) to give **15** (1.737 g, 65%) as a white foam as well as recovered starting material **14** (0.588 g, 28%). Data for **15**: R_f 0.3 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.90 (s, 1H, NH), 8.04 (d, 2H, J = 7.2 Hz, Ph), 7.55 (m, 1H, Ph), 7.37 (m, 2H, Ph), 7.27 (d, 1H, J = 8.1 Hz, H-6), 5.81 (d, 1H, J = 9.1 Hz, H-1′), 5.71 (d, 1H, J = 8.1 Hz, H-5), 5.61 (m, 1H, C*H*=CH$_2$), 5.06 (d, 1H, J = 17.1 Hz, CH=C*H*$_2$), 4.98 (d, 1H, J = 10.2 Hz, CH=C*H*$_2$), 4.69 (d, 1H, J = 6.2 Hz, H-3′), 4.55 (d, 1H, J = 12.2 Hz, CH$_2$OBz), 4.47 (d, 1H, J = 12.2 Hz, CH$_2$OBz), 3.80 (d, 1H, J = 11.8 Hz, H-5′), 3.69 (d, 1H, J = 11.8 Hz, H-5′), 3.26 (br s, 1H, OH), 2.75 (m, 1H, H-2′), 2.42 (m, 1H, C*H*$_2$CH=CH$_2$), 2.22 (m, 1H, C*H*$_2$CH=CH$_2$), 0.97 (s, 9H, SiC(CH$_3$)$_3$), 0.18 (s, 3H, SiCH$_3$), 0.14 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.2 (CO), 162.9 (C-4), 150.3 (C-2), 141.9 (C-6), 135.0 (*C*H=CH$_2$), 133.3 (Ph), 129.8 (Ph), 129.6 (Ph), 128.4 (Ph), 117.1 (CH=*C*H$_2$), 102.7 (C-5), 91.7, 87.3 (C-1′, C-4′), 74.7 (C-3′), 64.9, 63.7 (C-5′, CH$_2$O), 46.8 (C-2′), 30.2 (*C*H$_2$CH=CH$_2$), 25.9 (C(*C*H$_3$)$_3$), 18.2 (*C*(CH$_3$)$_3$), −4.3 (CH$_3$Si), −4.6 (CH$_3$Si); HRMALDI MS *m*/*z* (539.2192 [M + Na]$^+$, $C_{26}H_{36}O_7N_2Si$ − Na$^+$ calcd 539.2184).

Synthesis of 2′-Deoxy-2′-*C*-allyl-4′-*C*-hydroxymethyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)uridine (16). To a stirred solution of **15** (1.718 g, 3.33 mmol) in anhydrous DMF (50 mL) was added imidazole (1.714 g, 25.6 mmol) and TBSCl (1.691 g, 11.2 mmol). The mixture was stirred for 15 h. H_2O (60 mL) was added, and the mixture was extracted with Et_2O (3 × 150 mL). The combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (60 mL) and brine (60 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was dissolved in anhydrous CH_3OH (50 mL), and $NaOCH_3$ (0.773 g, 14.3 mmol) was added. The reaction mixture was stirred at 35 °C for 10 h and then at room temperature for 1 day. The reaction was quenched with 80% CH_3COOH (10 mL) and extracted with CH_2Cl_2 (3 × 100 mL), and the combined extracts were washed with a saturated aqueous solution of $NaHCO_3$ (60 mL), dried ($MgSO_4$), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc−petroleum ether 1:3 v/v) to give **16** (1.243 g, 71%) as a white foam: R_f 0.5 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (br s, 1H, NH), 7.60 (d, 1H, J = 8.2 Hz, H-6), 6.11 (d, 1H, J = 9.1 Hz, H-1′), 5.70 (dd, 1H, J = 2.1 Hz, J = 8.2 Hz, H-5), 5.60 (m, 1H, C*H*=CH$_2$), 5.03 (dd, 1H, J = 1.1 Hz, J = 17.0 Hz, CH=C*H*$_2$), 4.95 (d, 1H, J = 10.3 Hz, CH=C*H*$_2$), 4.42 (d, 1H, J = 5.7 Hz, H-3′), 3.87 (d, 1H, J = 12.1 Hz, C*H*$_2$OH), 3.78 (d, 1H, J = 10.7 Hz, H-5′), 3.71 (d, 1H, J = 10.7 Hz, H-5′), 3.52 (d, 1H, J = 12.1 Hz, C*H*$_2$OH), 2.44−2.30 (m, 2H, H-2′, C*H*$_2$CH=CH$_2$), 2.18 (m, 1H, C*H*$_2$CH=CH$_2$), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.13 (s, 3H, SiCH$_3$), 0.12 (s, 3H, SiCH$_3$), 0.10 (s, 3H, SiCH$_3$), 0.10 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.9 (C-4), 150.3 (C-2), 140.1 (C-6), 134.8 (*C*H=CH$_2$), 116.8 (CH=*C*H$_2$), 102.8 (C-5), 88.4, 87.5 (C-1′, C-4′), 75.3 (C-3′), 65.4, 63.7 (C-5′, CH$_2$O), 49.4 (C-2′), 29.3 (*C*H$_2$CH=CH$_2$), 25.9 (C(*C*H$_3$)$_3$), 25.8 (C(*C*H$_3$)$_3$), 18.3 (*C*(CH$_3$)$_3$), 18.1 (*C*(CH$_3$)$_3$), −4.3 (CH$_3$Si), −4.4 (CH$_3$Si), −5.5 (CH$_3$Si), −5.6 (CH$_3$Si); HRMALDI MS *m*/*z* (549.2776 [M + Na]$^+$, $C_{25}H_{46}O_6N_2Si_2$ − Na$^+$ calcd 549.2787).

Synthesis of 2′-Deoxy-2′-*C*-allyl-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-4′-*C*-vinyluridine (17). To a stirred solution of **16** (0.668 g, 1.27 mmol) in anhydrous CH_2Cl_2 (17 mL) was added Dess−Martin periodinane (0.659 g, 1.55 mmol). After 1 1/2 h, the mixture was filtered through Celite and the sinter was rinsed with EtOAc (100 mL). The combined organic phases were washed with a mixture of a saturated aqueous solution of $NaHCO_3$ and a saturated aqueous solution of $Na_2S_2O_3$ (1:1, 40 mL), and the water phase was extracted with CH_2Cl_2 (2 × 75 mL). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc−petroleum ether 1:1 v/v) to give the aldehyde (0.619 g, 93%) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.53 (s, 1H, CHO), 8.96 (s, 1H, NH), 7.77 (d, 1H, J = 8.1 Hz, H-6), 6.42 (d, 1H, J = 9.4 Hz, H-1′), 5.72 (d, 1H, J = 8.1 Hz, H-5), 5.64 (m, 1H, C*H*=CH$_2$), 5.05 (dd, 1H, J = 1.4 Hz, J = 17.1 Hz, CH=C*H*$_2$), 4.98 (d, 1H, J = 10.3 Hz, CH=C*H*$_2$), 4.50 (d, 1H, J = 5.0 Hz, H-3′), 4.04 (d, 1H, J = 11.4 Hz, H-5′), 3.68 (d, 1H, J = 11.4 Hz, H-5′), 2.51−2.14 (m, 3H, H-2′, C*H*$_2$CH=CH$_2$), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.90 (s, 9H, SiC(CH$_3$)$_3$), 0.14 (s, 3H, SiCH$_3$), 0.14 (s, 3H, SiCH$_3$), 0.07 (s, 3H, SiCH$_3$), 0.02 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.5 (CHO), 162.9 (C-4), 150.4 (C-2), 140.0 (C-6), 134.4 (*C*H=CH$_2$), 117.0 (CH=*C*H$_2$), 103.1 (C-5), 91.9, 89.3 (C-1′, C-4′), 77.9 (C-3′), 64.2 (C-5′), 49.4 (C-2′), 28.6 (*C*H$_2$CH=CH$_2$), 25.8 (C(*C*H$_3$)$_3$), 25.8 (C(*C*H$_3$)$_3$), 18.3 (*C*(CH$_3$)$_3$), 18.0 (*C*(CH$_3$)$_3$), −4.0 (CH$_3$Si), −4.7 (CH$_3$Si), −5.5 (CH$_3$Si), −5.6 (CH$_3$Si). A suspension of Ph_3PBrCH_3 (0.513 g, 1.44 mmol) in anhydrous THF (4 mL) was stirred at −78 °C under an Ar atmosphere, and BuLi (1.6 M in hexanes, 0.85 mL, 1.36 mmol) was added. The mixture was stirred for 15 min at −78 °C and then at 0 °C. A solution of the aldehyde (0.194 g, 0.37 mmol) in THF (2 mL) was added. The reaction mixture was stirred at 0 °C for 45 min, and after an additional 2 h at room temperature, a saturated aqueous solution of NH_4Cl (7 mL) was added. The resulting mixture was extracted with Et_2O (3 × 15 mL), and the combined extracts were washed with brine (10 mL), dried ($MgSO_4$), and evaporated under reduced pressure. Purification by silica gel column chromatography (EtOAc−petroleum ether 1:3 v/v) gave **17** (0.184, 95%) as a white foam: R_f 0.6 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.75 (s, 1H, NH), 7.80 (d, 1H, J = 8.2 Hz, H-6), 6.09 (d, 1H, J = 6.5 Hz, H-1′), 5.86 (dd, 1H, J = 10.9 Hz, J = 17.4 Hz, CC*H*=CH$_2$), 5.74−5.63 (m, 2H, H-5, CH$_2$C*H*=CH$_2$), 5.47 (dd, 1H, J = 1.7 Hz, J = 17.4 Hz, CCH=C*H*$_2$), 5.23 (dd, 1H, J = 1.7 Hz, J = 10.9 Hz, CCH=C*H*$_2$), 5.05−4.96 (m, 2H, CH$_2$CH=C*H*$_2$), 4.50 (d, 1H, J = 6.3 Hz, H-3′), 3.64−3.52 (m, 2H, H-5′), 2.40−2.29 (m, 2H, H-2′, C*H*$_2$CH=CH$_2$), 2.13 (m, 1H, C*H*$_2$CH=CH$_2$), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.93 (s, 9H, SiC(CH$_3$)$_3$), 0.11 (s, 3H, SiCH$_3$), 0.11 (s, 3H, SiCH$_3$), 0.10 (s, 3H, SiCH$_3$), 0.08 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0 (C-4), 150.1 (C-2), 140.5 (C-6), 135.6, 135.2 (*C*H=CH$_2$), 116.5, 116.1 (CH=*C*H$_2$), 102.6 (C-5), 89.0, 87.4 (C-1′, C-4′), 73.4 (C-3′), 66.4 (C-5′), 49.7 (C-2′), 30.5 (*C*H$_2$CH=CH$_2$), 25.9 (C(*C*H$_3$)$_3$), 25.9 (C(*C*H$_3$)$_3$), 18.3, 18.1 (*C*(CH$_3$)$_3$), −4.3 (CH$_3$Si), −4.4 (CH$_3$Si), −5.5 (CH$_3$Si), −5.5 (CH$_3$Si); MALDI MS *m*/*z* (547.2 [M + Na]$^+$, $C_{26}H_{46}O_5N_2Si_2$ − Na$^+$ calcd 547.2).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-*tert*-Butyldimethylsilyloxy-1-*tert*-butyldimethylsilyloxymethyl-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]-oct-2-ene (18). To a stirred solution of **17** (0.277 g, 0.53 mmol) in anhydrous CH_2Cl_2 (15 mL) was added Grubbs' second-generation

catalyst (((Mes)$_2$Im)(Cy$_3$P)Cl$_2$Ru=CHPh)[34] (0.022 g, 0.026 mmol), and the solution was heated to reflux for 25 h. The mixture was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc−petroleum ether 1:4 v/v) to give **18** (0.251 g, 96%) as a white foam: R_f 0.6 (EtOAc); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.25 (s, 1H, NH), 8.12 (d, 1H, J = 8.2 Hz, H-6), 5.91 (m, 1H, CH=C*H*CH$_2$), 5.65 (d, 1H, J = 8.2 Hz, H-5), 5.61 (s, 1H, H-1′), 5.41 (d, 1H, J = 9.3 Hz, C*H*=CHCH$_2$), 4.34 (d, 1H, J = 5.0 Hz, H-3′), 3.80 (d, 1H, J = 11.6 Hz, H-5′), 3.65 (d, 1H, J = 11.6 Hz, H-5′), 2.55 (m, 1H, CH=CHC*H*$_2$), 2.40−2.30 (m, 2H, H-2′, CH=CHC*H*$_2$), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.85 (s, 9H, SiC(CH$_3$)$_3$), 0.13 (s, 3H, SiCH$_3$), 0.12 (s, 3H, SiCH$_3$), 0.07 (s, 3H, SiCH$_3$), 0.06 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 163.5 (C-4), 150.2 (C-2), 140.2 (C-6), 130.7, 126.6 (CH=CH), 101.0 (C-5), 88.9, 81.8 (C-1′, C-4′), 65.1, 61.8 (C-3′, C-5′), 44.8 (C-2′), 28.5 (*C*H$_2$CH=CH), 25.9 (C(*C*H$_3$)$_3$), 25.5 (C(*C*H$_3$)$_3$), 18.3, 17.9 (*C*(CH$_3$)$_3$), −4.7 (CH$_3$Si), −5.1 (CH$_3$Si), −5.5 (CH$_3$Si), −5.6 (CH$_3$Si); HRMALDI MS *m/z* (517.2505 [M + Na]$^+$, C$_{24}$H$_{42}$O$_5$N$_2$Si$_2$ − Na$^+$ calcd 517.2525).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-Hydroxy-1-hydroxymethyl-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]oct-2-ene (9). A 1 M solution of TBAF in THF (1.1 mL, 1.1 mmol) was added to a stirred solution of **18** (0.247 g, 0.50 mmol) in anhydrous THF (9 mL), and after 3 h, an additional amount of the TBAF solution (0.14 mmol) was added. After stirring for another 2 h, the mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (petroleum ether, then CH$_3$OH (1−5%) in CH$_2$Cl$_2$) to give **9** (0.130 g, 98%) as a white solid: R_f 0.1 (2 × EtOAc); ^{1}H NMR (300 MHz, DMSO) δ 11.27 (s, 1H, NH), 7.96 (d, 1H, J = 8.1 Hz, H-6), 5.82 (m, 1H, CH=C*H*CH$_2$), 5.55 (d, 1H, J = 8.1 Hz, H-5), 5.48 (d, 1H, J = 9.6 Hz, C*H*=CHCH$_2$), 5.41 (s, 1H, H-1′), 5.15 (d, 1H, J = 4.8 Hz, 3′-OH), 5.09 (t, 1H, J = 5.4 Hz, 5′-OH), 4.12 (t, 1H, J = 4.8 Hz, H-3′), 3.59 (dd, 1H, J = 5.4 Hz, J = 12.5 Hz, H-5′), 3.50 (dd, 1H, J = 5.4 Hz, J = 12.5 Hz, H-5′), 2.47 (m, 1H, CH=CHC*H*$_2$), 2.31 (m, 1H, H-2′), 2.08 (m, 1H, CH=CHC*H*$_2$); ^{13}C NMR (75 MHz, DMSO) δ 163.4 (C-4), 150.3 (C-2), 140.2 (C-6), 130.2, 127.3 (CH=CH), 100.5 (C-5), 88.0, 81.2 (C-1′, C-4′), 64.4, 60.4 (C-3′, C-5′), 43.7 (C-2′), 28.2 (*C*H$_2$-CH=CH); HRMALDI MS *m/z* (289.0795 [M + Na]$^+$, C$_{12}$H$_{14}$O$_5$N$_2$ − Na$^+$ calcd 289.0795).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-(Hydroxy)-1-(hydroxymethyl)-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]octane (8). To a stirred solution of **9** (60 mg, 0.23 mmol) in CH$_3$OH (1 mL) was added PtO$_2$ (21 mg, 0.09 mmol). The mixture was degassed with N$_2$ and then placed under a hydrogen atmosphere. The reaction mixture was stirred for 1 1/2 h and then filtered through Celite. The filtrate was evaporated under reduced pressure to give **8** (62 mg, 100%) as a white foam: R_f 0.1 (EtOAc); ^{1}H NMR (300 MHz, DMSO) δ 11.24 (s, 1H, NH), 8.36 (d, 1H, J = 8.0 Hz, H-6), 5.63 (s, 1H, H-1′), 5.53 (d, 1H, J = 8.0 Hz, H-5), 5.25−5.18 (m, 2H, OH), 4.07 (m, 1H, H-3′), 3.48−3.43 (m, 2H, H-5′), 2.22 (m, 1H, H-2′), 1.90 (m, 1H, CH$_2$), 1.68−1.14 (m, 5H, CH$_2$); ^{13}C NMR (75 MHz, DMSO) δ 163.4 (C-4), 150.2 (C-2), 140.4 (C-6), 99.7 (C-5), 86.5, 86.0 (C-1′, C-4′), 64.2, 61.6 (C-3′, C-5′), 44.9 (C-2′), 25.5, 23.0, 17.4 (CH$_2$-CH$_2$CH$_2$); HRMALDI MS *m/z* (291.0951 [M + Na]$^+$, C$_{12}$H$_{16}$O$_5$N$_2$ − Na$^+$ calcd 291.0951).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]oct-2-ene (19). DMTCl (76 mg, 0.22 mmol) was added to a stirred solution of **9** (48 mg, 0.18 mmol) in anhydrous pyridine (0.5 mL) and anhydrous CH$_3$CN (0.5 mL), and the mixture was stirred for 22 h. Additional DMTCl (13 mg, 0.04 mmol) was added, and after stirring for 1 h, the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (CH$_3$OH (0−5%), pyridine (1%) in CH$_2$Cl$_2$) to give the DMT-protected product (79 mg, 78%) as a colorless oil: R_f 0.3 (EtOAc); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.62 (s, 1H, NH), 8.20 (d, 1H, J = 8.0 Hz, H-6), 7.46−7.19 (m, 9H, Ar), 6.85 (dd, 4H, J = 8.9 Hz, J = 1.3 Hz, Ar), 5.99 (m, 1H, CH=C*H*CH$_2$), 5.64 (s, 1H, H-1′), 5.48−5.45 (m, 2H, H-5, C*H*=CHCH$_2$), 4.50 (dd, 1H, J = 5.6 Hz, J = 7.7 Hz, H-3′), 3.80 (s, 6H, OCH$_3$), 3.54 (d, 1H, J = 11.1 Hz, H-5′), 3.36 (d, 1H, J = 11.1 Hz, H-5′), 2.64−2.54 (m, 2H, CH=CHC*H*$_2$, H-2′), 2.43 (m, 1H, CH=CHC*H*$_2$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 163.6 (C-4), 158.6 (Ar), 150.3 (C-2), 144.5 (Ar), 140.1 (C-6), 135.4, 135.3, 131.4, 130.1, 128.1, 128.0, 127.2, 127.1, 113.3 (Ar, CH=CH), 101.2 (C-5), 89.2, 86.9, 81.0 (C-1′, C-4′, Ar$_3$*C*), 66.0, 62.2 (C-3′, C-5′), 55.2 (OCH$_3$), 44.2 (C-2′), 28.0 (CH=CH*C*H$_2$). To a stirred solution of the DMT-protected intermediate (74 mg, 0.13 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) was added *N*,*N*-diisopropylethylamine (0.1 mL, 0.58 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.05 mL, 0.22 mmol). The mixture was stirred for 19 h, and then we added CH$_2$Cl$_2$ (4 mL). The mixture was washed with a saturated aqueous solution of NaHCO$_3$. The aqueous phase was extracted with CH$_2$Cl$_2$ (5 mL), and the combined organic phases were dried (Na$_2$SO$_4$) and evaporated under reduced pressure. Purification by silica gel column chromatography (EtOAc−petroleum ether 1:4 v/v with 1% NEt$_3$) gave **19** (57 mg, 57%) as a white foam: R_f 0.4 (EtOAc); ^{31}P (CDCl$_3$) δ 149.57.

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]octane (20). To a stirred solution of **8** (62 mg, 0.23 mmol) in anhydrous CH$_2$Cl$_2$ (1 mL) was added Et$_3$N (0.07 mL, 0.50 mmol) and DMTCl (83 mg, 0.24 mmol). The mixture was stirred for 4 h and then evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give the DMT-protected product (101 mg, 78%) as a white foam: R_f 0.4 (EtOAc); ^{1}H NMR (300 MHz, CDCl$_3$) δ 8.38 (d, 1H, J = 8.0 Hz, H-6), 7.99 (br s, 1H, NH), 7.46−7.12 (m, 9H, Ar), 6.88−6.78 (m, 4H, Ar), 5.78 (s, 1H, H-1′), 5.33 (d, 1H, J = 8.0 Hz, H-5), 4.25 (m, 1H, H-3′), 3.80 (s, 6H, OCH$_3$), 3.51 (d, 1H, J = 11.4 Hz, H-5′), 3.28 (d, 1H, J = 11.4 Hz, H-5′), 2.42 (m, 1H, H-2′), 1.88 (m, 1H, CH$_2$), 1.75−1.50 (m, 3H, CH$_2$), 1.30−1.15 (m, 2H, CH$_2$); HRESI MS *m/z* (593.2254 [M + Na]$^+$, C$_{33}$H$_{34}$O$_7$N$_2$ − Na$^+$ calcd 593.2258). To a stirred solution of the DMT-protected intermediate (100 mg, 0.18 mmol) in anhydrous CH$_2$Cl$_2$ (1.2 mL) was added *N*,*N*-diisopropylethylamine (0.11 mL, 0.64 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.06 mL, 0.25 mmol). After 4 1/2 h, more 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.03 mL, 0.13 mmol) was added, and after stirring for 6 h, CH$_2$Cl$_2$ (10 mL) was added. The mixture was washed with a saturated aqueous solution of NaHCO$_3$ (8 mL), and the aqueous phase was extracted with CH$_2$Cl$_2$ (10 mL). The combined organic phases were dried (Na$_2$SO$_4$) and evaporated under reduced pressure. Purification by silica gel column chromatography (EtOAc−petroleum ether 1:4 v/v with 0.5% pyridine) gave **20** (85 mg, 63%) as a white foam: R_f 0.5, 0.6 (EtOAc); ^{31}P (CDCl$_3$) δ 150.42, 149.54; HRESI MS *m/z* (793.3330 [M + Na]$^+$, C$_{42}$H$_{51}$O$_8$N$_4$P − Na$^+$ calcd 793.3337).

Synthesis of Oligodeoxynuclotides. Oligonucleotide synthesis was carried out on an automated DNA synthesizer following the phosphoramidite approach. Synthesis of oligonucleotides **21**−**25** was performed on a 0.2 μmol scale by using the amidites **19** and **20** as well as the corresponding commercial 2-cyanoethyl phosphoramidites of the natural 2′-deoxynucleosides. The synthesis followed the regular protocol for the DNA synthesizer. However, for **19** and **20**, a prolonged coupling time of 10 min was used. Coupling yields for all 2-cyanoethyl phosphoramidites were >98%. After a final detritylation, the oligonucleotides were removed from the universal solid support by treatment with concentrated ammonia at 55 °C for 20 h affording the >95% pure oligonucleotides. MALDI-MS [M − H]$^-$ gave the following results (found/calcd): **22** (2776.0/2780); **23** (2827.8/2828); **24** (2775.7/2778); **25** (2822.8/2826).

Melting Experiments. UV melting experiments were carried out on a UV spectrometer. Samples were dissolved in a medium salt buffer containing Na$_2$HPO$_4$ (5 mM), NaH$_2$PO$_4$ (10 mM), NaCl (100 mM), and EDTA (0.1 mM) (pH 7.0) with 1.5 μM concentrations of the two complementary sequences. The increase in

absorbance at 260 nm as a function of time was recorded while the temperature was increased linearly from 10 to 70 °C at a rate of 0.5 °C/min by means of a Peltier temperature programmer. The melting temperature was determined as the local maximum of the first derivatives of the absorbance vs temperature curve. All melting curves were found to be reversible. All determinations are averages of triplicates.

CD Spectroscopy. CD spectra were recorded at the Department of Chemistry, University of Copenhagen. The same medium salt buffer as that used in the UV melting experiments was used with 3.0 μM concentrations of the two complementary sequences.

Quantum Mechanical Calculations. Ab initio quantum mechanical calculations were performed with the Gaussian 03 program.[38] Full geometry optimizations (HF/6-31G*) were carried out for the selected nucleosides as mentioned in the text. To ensure that no hydrogen bonds were formed between the 3′-hydroxy group and the nucleobase, the ϵ torsion angle was constrained to $-75°$. The β, γ, and χ angles were not constrained, and in the optimized structures, there are no steric interactions among the 5′-hydroxy group, the sugar, and the nucleobase.

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Supporting Information Available: The general introduction to the Experimental Section as well as the experimental details for the compounds **11** and **12**. This material is available free of charge via the Internet at http://pubs.acs.org.

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