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Synthesis of Functionalized Carbocyclic Locked Nucleic Acid Analogues by Ring-Closing Diene and Enyne Metathesis and Their Influence on Nucleic Acid Stability and Structure

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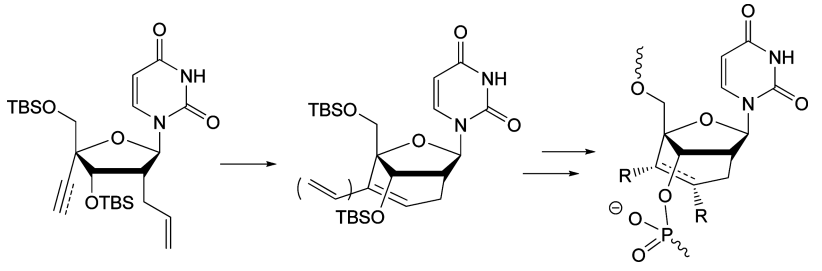

A series of bicylic 2′-deoxynucleosides that are locked in the *N*-type conformation due to three-carbon linkages between the 2′- and 4′-positions have been prepared by ring-closing diene or enyne metathesis. The alkene or 1,3-diene hereby introduced in the bicyclic system is further derivatized, the latter showing the expected potential for Diels−Alder reactions. Four derivatives that are saturated or unsaturated as well as functionalized at the 2′−4′-linkage are incorporated into oligodeoxynucleotides, and the affinity of these for complementary RNA and DNA is studied. Substantially increased affinity for complementary RNA is observed, especially with additional hydroxyl groups attached to the bicyclic system. On the other hand, decreased affinity for complementary single-stranded DNA is obtained, whereas only a very small influence on a triplex-forming oligonucleotide sequence is found. Hence, a strong RNA-selective nucleic acid recognition is seen, and it can be concluded that the 2′-oxygen atom is less important for the formation of DNA:RNA duplexes than for the formation of DNA:DNA duplexes. However, the lack of a 2′-oxygen in the duplex formation can be partly compensated by other hydrophilic moieties around the 2′−4′-linkages indicating structural water binding to be of significant importance.

Introduction

Oligonucleotides that are conformationally restricted due to bicyclic nucleoside building blocks[1] have demonstrated unique molecular recognition of complementary nucleic acid sequences and thus very promising properties as, e.g., antisense oligonucleotides (AOs),[2] siRNA,[3] or triplex-forming oligonucleotides (TFOs).[4] More than any other nucleic acid

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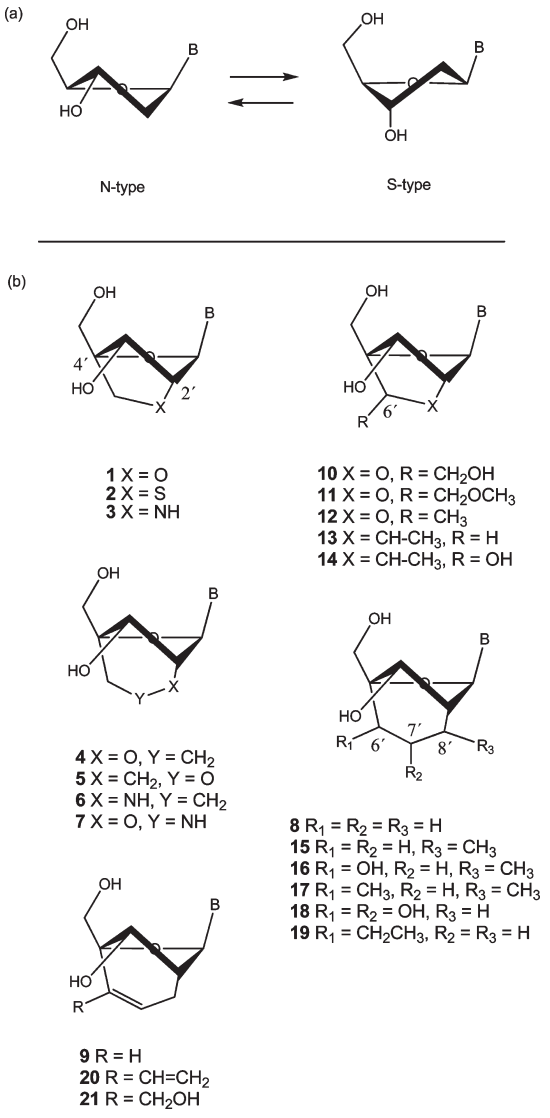

FIGURE 1. (a) Low energy conformations of 2′-deoxynucleotides. (b) Bicyclic nucleosides with 2′−4′-linkages and locked *N*-type conformations. B = a nucleobase.

analogue, locked nucleic acid (LNA) has been recognized as the prime tool for engineering strong and specific nucleic acid recognition.[5,6] LNA constitutes only a small structural perturbation to natural nucleic acids, and its preparation is completely compatible with standard solid-phase DNA synthesis. The LNA nucleoside monomer (Figure 1, **1**) is a bicyclic nucleoside that is locked in an *N*-type conformation due to an oxymethylene bridge between the 2′- and 4′-positions. By introducing one or more LNA−nucleoside monomers into an otherwise unmodified oligodeoxynucleotide, unprecedented recognition of complementary RNA and DNA has been obtained. The increase in thermal stability (ΔT_m) of the formed duplexes compared to unmodified duplexes ranges from +3 to +8 °C for each incorporation of an LNA monomer.[5,6] It has been demonstrated that each LNA−nucleoside monomer is able to conformationally tune its neighboring unmodified 2′-deoxynucleosides from *S*- to *N*-type conformations (Figure 1).[7] By this means, the overall duplex conformation is driven toward A-type or A-type-like duplex forms by the introduction of only a few LNA nucleosides.

The success of LNA has motivated the development of an increasing number of chemical analogues of the original LNA. The first were the thio and amino analogues **2** and **3** that demonstrated recognition properties almost comparable to those of LNA.[8] The ΔT_m's for each modification in an oligodeoxynucleotide range between +3 and +5 °C with DNA complements and between +4 and +8 °C with RNA complements. Furthermore, the amino group of amino-LNA, **3**, has been used as an attachment point for a large variety of substituents organizing these on the rim of the A-type duplex.[9] The first analogue with a longer 2′−4′-bridge was ENA, **4**, demonstrating almost the same stabilization of nucleic acid duplexes formed with complementary RNA (ΔT_m's between +3.5 and +5.5 °C in a mixed sequence context).[10,11] With complementary DNA **4** shows only a small stabilization (ΔT_m's between +0.5 and +2 °C).[10,11] The analogue, in which the oxygen is positioned in the neighboring position, **5**, demonstrated a slightly lower affinity for both complementary RNA (ΔT_m's between +2 and +3 °C) and complementary DNA (ΔT_m's between −0.5 and +1 °C).[12] Also, the amino analogue of ENA, **6**, has been studied and found to give similar affinities for RNA (ΔT_m's between +2.5 and +4 °C) and even lower for DNA (ΔT_m's between −0.5 and −3 °C).[13] More recently, also the oxamine analogue **7** (as well as its *N*-methylated and benzylated derivatives) has been introduced.[14] In the sequences studied, the affinities for both RNA and DNA were surprisingly good and almost similar to the results of LNA with ΔT_m's between +5.3 and +6.3 °C with complementary RNA and between +1.0 and +3.8 °C with complementary DNA.[14] Even longer 2′−4′-bridges have been introduced, however, leading only to affinities for complementary RNA comparable to what is found for unmodified oligodeoxynucleotides and slightly decreased affinities for complementary DNA.[11,15]

In order to study the effect of constitution of the 2′−4′-linkage and the importance of a 2′-oxygen for duplex

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formation, we introduced the first carbocyclic analogues of ENA, **8**, with a saturated ring, and **9** (B = uracil) containing an unsaturated cyclohexene ring (Figure 1).[16] The synthesis was based on ring-closing metathesis as the key step toward the bicyclic system. When incorporated into oligodeoxynucleotides, both **8** and **9** demonstrated increased stability of DNA:RNA hybrid duplexes (ΔT_m's between +2.5 and +5.0 °C per modification) comparable to what was found for ENA sequences, whereas the stability of dsDNA duplexes was destabilized (ΔT_m ranges between −2.5 and −1.0 °C per modification) opposite to what was found for ENA. CD spectroscopy revealed that the bicyclic nucleosides induced formation of A-type like duplexes, albeit to a lesser degree than found for LNA monomers.[16]

Recently, a series of branched analogues of LNA have been introduced. We introduced a 6′-branch by using a stereoselective mercury cyclization to give the 6′(*R*)-hydroxymethyl-LNA derivative **10** (B = thymine),[17] whereas Seth et al. synthesized the derivatives **11** and **12** after separation of 6′-epimers (B = cytosine/uracil) and studied these in oligonucleotides rendering improved RNA recognition in end-modified sequences when compared to unmodified or MOE-modified sequences but slightly decreased RNA recognition when compared to corresponding LNA sequences.[18] Very recently, Chattopadhyaya and co-workers continued the study of electrostatic effects around the 2′−4′-linkage by the introduction of a number of carbocyclic derivatives as represented by **13**−**17** (B = thymine), with a variety of substitutions for the 6′-position but all with a methyl group at the 7′-position (**13**, **14**) or 8′-position (**15**−**17**) pointing into the minor groove.[19,20] The 6′-unsubstituted derivatives **13** (mixture of 7′-epimers) and **15** (8′(*S*)-configuration) were reported first,[19] and oligonucleotides with single incorporations demonstrated enhanced affinities for RNA, more pronounced for the LNA analogues **13** (ΔT_m's between +2.5 and +4.0 °C) than for the ENA analogue **15** (ΔT_m's between +0.5 and +1.5 °C).[20] Like with unbranched carbocyclic analogues **8** and **9**, the affinity for complementary DNA is decreased, more so for **15** (ΔT_m's between −1.5 and −5.5 °C) than for **13** (ΔT_m's between +0.5 and −2.5 °C).[19] The carbocyclic LNA derivative **14** has been studied as all four 6′/7′-stereoisomeric combinations separately and together with 6′-methylated derivatives, whereas the carbocyclic ENA derivatives **16** and **17** have been studied as the two different 6′-epimers with fixed 8′(*S*)-configuration. In general, only very small changes of the RNA affinities (approximately ±1 °C) as compared to the data for **13** and 15, respectively, were determined.[20] Concerning the DNA affinities, larger changes (mostly decreases) were found, and as the two extreme examples, the 6′(*S*)-postitioned OH group of **14** drives the DNA affinity upward compared to **13** to be generally slightly higher that for an unmodified sequence (ΔT_m's between −1.0 and +2.5 °C), whereas the 6′(*S*)-positioned CH_3 group of **17** drives the DNA affinity further down as compared to **15** (ΔT_m's between −3.0 and −8.5 °C).[20]

Among the members of this series of 2′−4′-bridged locked nucleic acid analogues, several have been studied as building blocks in triplex-forming oligonucleotides (TFOs). TFOs are short oligonucleotides designed to target dsDNA duplexes with purine tracts by forming Hoogsteen-type base-pairing parallel to the purine-rich strand.[4] Incorporation of LNA monomers, **1**, into the TFOs led to significant increases in thermal stability of the triplexes with ΔT_m's up to +10 °C reported,[21] although in most other sequences the change around +5 °C.[22] However, the ability to form triplexes disappears completely with fully modified LNA sequences.[22] ENA, **4**, has shown almost the same increases in triplex stability, and as opposed to LNA, fully modified ENA sequences also form stable triplexes.[23] Amino-LNA, **3**, shows the same effect as LNA in partly modified TFOs.[21] With various N-substituents, even more pronounced increases in affinity have been observed.[21] However, the introduction of the carbocyclic analogue **8** in TFOs has shown only almost neutral influence on the triplex stabilities (ΔT_m's between −0.5 and +1.3 °C).[21] The incorporation of **7**, on the other hand, demonstrated very high increases in affinity for the dsDNA target with ΔT_m's between +3 and +11 °C. This very positive effect is suggested to be due to a hydrogen bond between the NH in the bridge of the bicyclic nucleoside and its 3′-*O*-phosphate.[14]

All of the mentioned locked nucleosides with 2′−4′-bridges are locked in perfect *N*-type conformations as validated by their pseudorotation angles, *P* (obtained from NMR, X-ray data and/or modeling), grouping in the narrow spectrum around 12−27°.[16,24,25] The puckering amplitude, ν_{max},[24] on the other hand, follows the number of atoms in the 2′−4′-bridge; LNA and other analogues with two-atom bridges have ν_{max} in the range of 56−58°,[5,11,16,25] ENA and other analogues with three atom-bridges have ν_{max} around 46−48°,[11,16,25] and the nucleosides with even longer four-atom bridges have ν_{max} around 38°.[15,16] As excellent hybridization behavior of the corresponding oligonucleotides has been found with the use of both two- and three-atom bridges, the differences should also be found in the constitution of the bridge, for instance in the heteroatoms present as well as in the electrostatic surroundings as influenced by substituents on the 2′−4′-bridge.

Herein, we contribute to this study by the introduction of four new analogues of the carbocyclic derivatives **8** and **9** with either hydrophobic or hydrophilic substituents at the

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6′- or 7′-positions but with free unsubstituted 8′-positions, **18**−**21** (B = uracil). The preparation of these is based on the ring-closing metathesis technique formerly giving **8** and **9**[16] but this time being elaborated into ring-closing enyne metathesis leading to the 1,3-diene system of compound **20**, which has great potential for further derivatization and so has been processed into **19** and **21**.

Results

Chemical Synthesis of Bicyclic Nucleosides. The ring-closing metathesis (RCM) has become an excellent tool for the synthesis of medium and large ring systems.[26] In the field of nucleosides, the use of RCM reactions in the preparation of conformationally restricted bi- and tricyclic nucleosides[16,27] as well as di- and trinucleotides[28] has been reported by us and others.[29] The synthesis of the bicyclic nucleosides **8** and **9** (B = uracil) was performed from uridine, which was converted in nine synthetic steps to the 2′-deoxy-2′-allyl-4′-hydroxymethyluridine derivative **22** (Scheme 1).[16] Oxidation and Wittig methylenation afforded **23**, which was converted by RCM using Grubbs' second-generation catalyst to **24** affording after deprotection **9** and, thereafter, by hydrogenation the saturated bicyclic nucleoside **8**.[16] The cyclohexene of **9** is an obvious point for further activation of the bicyclic skeleton, and we decided to investigate the introduction of hydrophilic moieties around the 2′−4′-bridge. Therefore, the protected bicyclic nucleoside **24** was treated with OsO_4 to give the dihydroxy derivative **25** in 79% yield as a single stereoisomer (Scheme 1). The 6′(*S*),7′(*S*)-configuration of **25** was proven by NMR (see below for the analysis of both configuration and conformation). In order to make a derivative that is appropriately protected to be a building block for standard automated solid-phase oligonucleotide synthesis using the phosphoramidite approach, the free hydroxyl groups were protected as acetate esters to give **26** in 93% yield. Then, the silyl groups were removed with TBAF to give the deprotected compound **27**, and the 5′-hydroxyl group was selectively protected as a 4,4′-dimethoxytrityl (DMT) ether giving **28** in 34% overall yield. Finally, the 3′-*O*-phosphoramidite **29** was obtained in quantitative yield.

Whereas ring-closing diene metathesis is an established technique also in the preparation of various nucleoside derivates,[27−29] the ring-closing enyne metathesis is less used, and only a single bicyclic nucleoside derivative prepared by this method has been presented.[30] In order to convert the key intermediate **22**[16] into an appropriate substrate for enyne metathesis, **22** was oxidized using the Dess−Martin periodinane to give an aldehyde, which was further converted to the alkyne **30** in 65% yield by the use of the so-called Bestmann−Ohira reagent.[31] The enyne derivative **30** was reacted with Grubbs' second-generation catalyst using microwave heating and provided the enyne metathesis 1,3-diene product **31** in 82% yield. Removal of the silyl groups was accomplished in 71% yield with potassium fluoride and crown ether to give the unprotected bicyclic nucleoside **20**, which was selectively protected with the DMT-group to give the intermediate **32** in 65% yield. Phosphitylation was achieved to give the 3′-*O*-phosphoramidite **33** in 60% yield.

Complete hydrogenation of the conjugated double bonds of **31** was achieved by the use of Adams' catalyst to give a mixture of diastereomers **19** in 70% yield as an 8:1 ratio. Simple modeling in combination with the experience from the stereoselective dihydroxylation on **24** strongly indicates the favor of the 6′(*R*)-isomer. Due to significant overlap of signals, however, no proof for this was given by NMR. Again, a selective tritylation of the 5′-OH was performed giving **34** in 77% yield, and phosphitylation afforded the 3′-*O*-phosphoramidite **35** in 48% yield.

Finally, we decided to convert the hydrophobic 6′-substituent of **20** into a hydrophilic hydroxymethyl moiety by selective oxidative cleavage of the terminal double bond of the 1,3-diene substrate. Catalytic amounts of OsO_4 with *N*-methlymorpholine-*N*-oxide as the cooxidant have been reported to give moderate selectivity for similar substrates,[32] but with reference to even better selectivity,[33] we used the sharpless AD-mix reagent for selective dihydroxylation of the terminal double bond of **31** followed by oxidative cleavage with $NaIO_4$ to give the desired α,β-unsaturated aldehyde. Selective reduction of the carbonyl group was achieved using Luche conditions ($NaBH_4/CeCl_3$) to give the hydroxymethyl derivate **36** in 54% yield over the three steps. The primary hydroxy group was protected as its benzoate ester **37**, and the silyl protecting groups were removed with potassium fluoride and crown ether to give compound **38** in 41% yield. The 5′-OH group was selectively protected with the DMT group, and the resulting compound **39** was converted into the 3′-*O*-phosphoramidite **40** in 45% yield.

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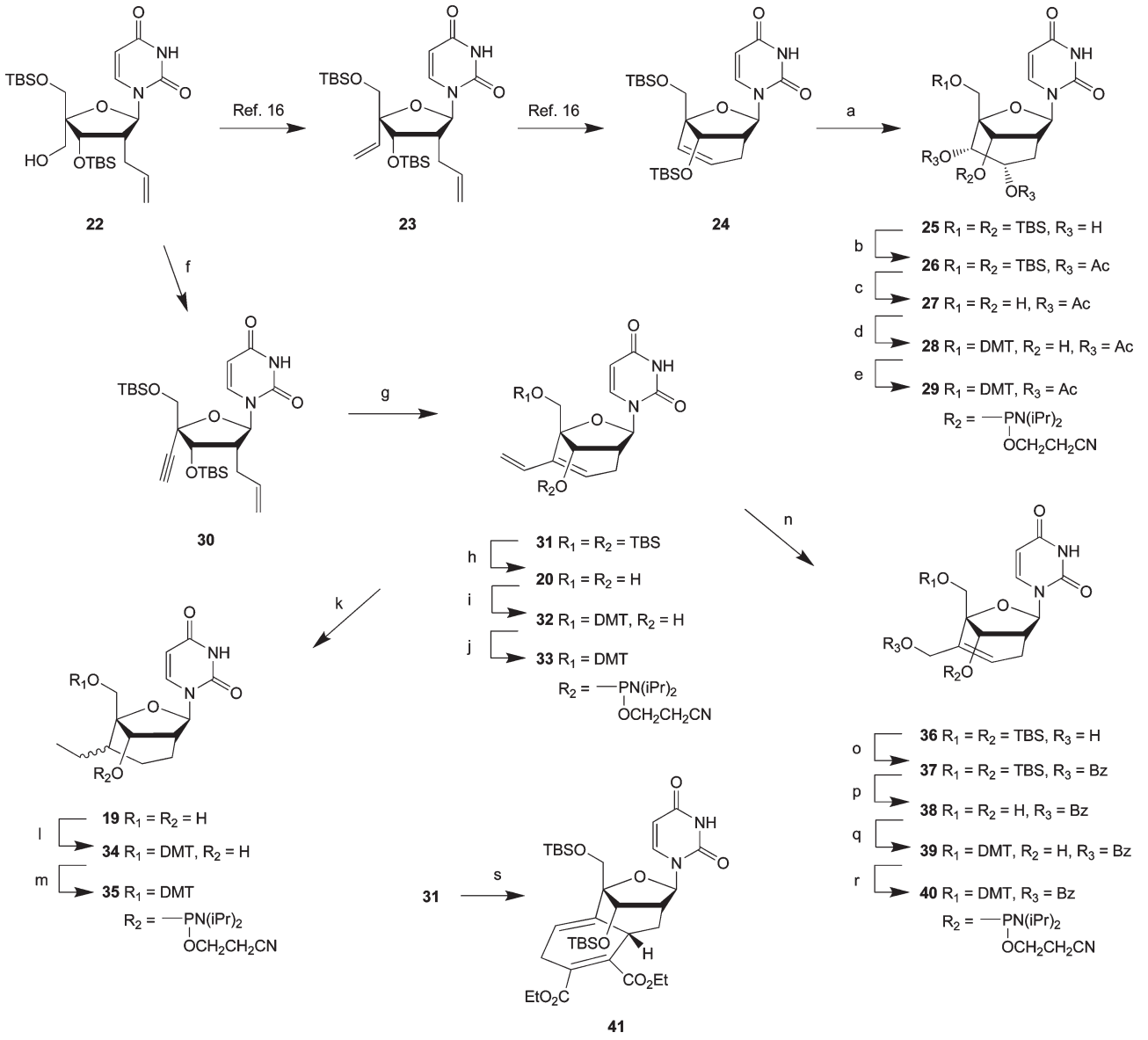
SCHEME 1. Synthesis of Bi- and Tricyclic Nucleosides[a]



[a]Reagents and conditions: (a) OsO_4/NMO, THF, H_2O, 100 °C, 79%; (b) Ac_2O, pyridine, DMAP, 93%; (c) TBAF, THF, 42%; (d) DMT-Cl, pyridine, CH_3CN, 79%; (e) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, DCE, 100%; (f) (i) Dess−Martin periodinane, CH_2Cl_2, (ii) dimethyl 2-oxopropylphosphonate, TsN_3, K_2CO_3, CH_3OH, CH_3CN, 65%; (g) Grubbs' second-generation catalyst, CH_2Cl_2, 100 °C, MW, 82%; (h) KF, 18-crown-ether-6, CH_3CN, 100 °C, MW, 71%; (i) DMT-Cl, pyridine, CH_3CN, 65%; (j) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, 60%; (k) H_2, PtO_2, CH_3OH, 70%; (l) DMT-Cl, pyridine, CH_3CN, 72%; (m) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, 48%; (n) (i) K_3FeCN_6, K_2CO_3, $K_2OsO_4 \cdot 2H_2O$, $(DHQ)_2PHAL$, Na_2SO_3, *t*-BuOH, H_2O, (ii) $NaIO_4$, THF, H_2O, (iii) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, CH_3OH, 54%; (o) BzCl, pyridine, 53%; (p) KF, 18-crown-ether-6, CH_3CN, 100 °C, MW, 41%; (q) DMT-Cl, pyridine, CH_3CN, 77%; (r) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, 45%; (s) $EtO_2CC{\equiv}CCO_2Et$, toluene, 150 °C, MW, 75%. $(DHQ)_2PHAL$ = hydroquinine 1,4-phthalazinediyl diether, Ts = 4-toluenesulfonyl, DMT = 4,4′-dimethoxytrityl, NMO = *N*-methylmorpholine *N*-oxide, DCE = 1,2-dichloroethane.

Finally, the bicyclic nucleoside **20** with its 1,3-diene moiety is an obvious substrate for Diels−Alder reactions and therefore an easy access to further derivatization. As Diels−Alder reactions have previously been demonstrated to work on oligonucleotides with 1,3-dienes in the 3′-end,[34] also oligonuncleotides contacting **20** should be potential substrates for future studies. We decided to make a simple proof of principle for the nucleoside, and we reacted the protected compound **31** with an alkyne (diethyl acetylenedicarboxylate) and achieved the tricyclic nucleoside **41** as a single stereoisomer in 75% yield (see Scheme 1 for configurational analysis).

Configurational and Conformational Analyses. The configuration of **25** was determined from ROE contacts (Figure 2). Contacts between H-1′ and OH-7′, between H-1′ and H-8′$_a$ (hereafter defined to be placed below the furanose ring), and between H-6′ and H-8′$_b$ unequivocally shows that the configuration of C-6′ and C-7′ is (*S*,*S*). We explored the conformation of the unprotected form of **25** with ab initio calculations. In the low energy conformation (Figure 2),

(34) Hill, K. W.; Taunton-Rigby, J.; Carter, J. D.; Kropp, E.; Vagle, K.; Pieken, W.; McGee, D. P. C.; Husar, G. M.; Leuck, M.; Anziano, D. J.; Sebesta, D. P. *J. Org. Chem.* **2001**, *66*, 5352–5358.

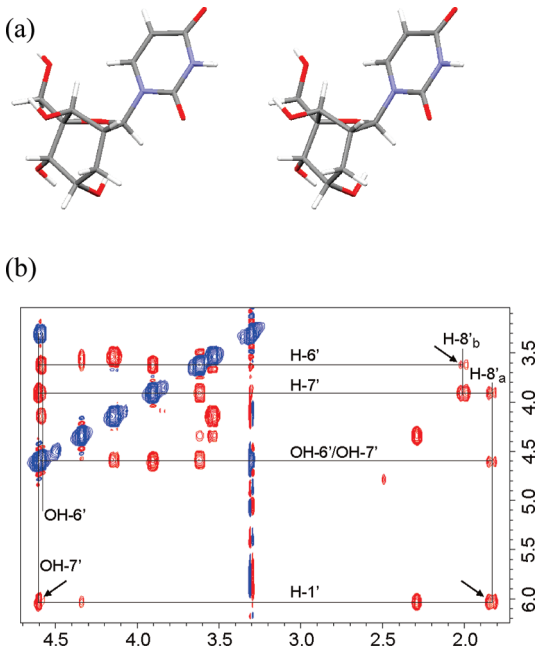


FIGURE 2. (a) Stereoview of the lowest energy conformation of an unprotected form of **25** (i.e., compound **18** (B = U)) and (b) ROESY spectrum of compound **25**. For atom numbering, see Figure 1.

the pseudoration angle P of the sugar ring is 7° and the puckering amplitude ν_{max} is 46°. The C3′-*endo* sugar conformation is in accordance with vanishing H-1′−H-2′ scalar coupling constants. The six-membered ring connecting C-2′ and C-4′ adopts a chair conformation with C-3′ displaced slightly more than C-7′ from the plane formed by the remaining four atoms in the ring. To gauge how constrained the bicyclic ring system of **25** is, we calculated the energy difference between the chair and boat conformations of the six-membered ring. The sugar ring possesses very little freedom in conformational space being locked in a C3′-*endo* conformation. In the boat conformation, C-7′ is displaced only very slightly from the plane formed by C-2′, C-8′, C-6′, and C-4′. It appears that further displacement of C-7′, providing a proper boat conformation, would lead to a steric clash between H-7′ and O-3′. Accordingly, the boat conformation is rather unfavorable, and its energy is 8.7 kcal/mol higher than that of the chair conformation. The completely planar conformation is only 0.3 kcal/mol higher in energy than the boat conformation. Thus, the unprotected form of **25** appears quite constrained in the conformation shown in Figure 2.

In addition, the configuration of **41** was determined by a ROESY spectrum. The ROE contact between H-1′ and H-8′$_a$ assigned H-8′$_a$ to be placed below the furanose ring and therefore H-8′$_b$ in the opposite position. Hereafter, the contact between H-7′ and H-8′$_b$ and the lack of contact between H-7′ and H-8′$_a$ unequivocally show that the configuration of C-7′ is (*S*) as shown in Scheme 1.

Synthesis and Hybridization Properties of Oligonucleotides. In order to study the affinity for complementary nucleic acid DNA- and RNA-sequences with an easy comparison, we used the same 9-mer oligodeoxynucleotide sequence as used in our former study on the bicyclic nucleosides **8** and **9**[16] as well as in the original studies on LNA[5] and other nucleic acid analogues.[8,35] Eight different modified 9-mer oligonucleotides were synthesized on solid support by using an automated DNA synthesizer. The four modified 3′-*O*-phosphoramidites of the present study, **29**, **33**, **35**, and **40** (incorporating **18**−**21**), were all coupled in good overall yields in combination with commercially available 2′-deoxynucleoside 3′-*O*-phosphoramididtes. 1*H*-Tetrazole was used as the activating agent for the phosphoramidites **33** and **35**, whereas pyridinium hydrochloride was used as the activator for **29** and **40**. A 30 min coupling time was used for the modified amidites. By the end of the oligonucleotide syntheses, deprotection of all base-labile protecting groups including the acetate and benzoate esters from **29** and **40**, respectively, as well as cleavage from the solid support was achieved by the use of 32% aqueous ammonia. The composition and purity of all modified oligonucleotides was confirmed by MALDI MS and ion exchange chromatography/RF-HPLC, respectively.

The hybridization of the modified oligonucleotides toward complementary DNA and RNA was studied by thermal denaturation experiments. The melting temperatures (T_m) of the modified duplexes were determined and compared with unmodified duplexes (Table 1). The sequence, **43**, with one incorporation of the nucleoside **18** demonstrated a melting temperature of the duplex formed with complementary RNA that is increased with 5.0 °C as compared to the unmodified duplex formed between **42** and RNA. This increase in duplex stability is the same for one incorporation of **21** (sequence **49**, ΔT_m = 4.9 °C), whereas the stability increase for the DNA:RNA duplexes with one incorporation of either **19** or **20** (sequences **45** and **47**) is slightly less pronounced (+3.8 and +3.2 °C, respectively). With the incorporation of three modified nucleosides in the DNA:RNA duplex, the increase in stability per modification is in all cases slightly smaller than with one incorporation, i.e., between +2.1 and +4.7 °C, with the most stable of all the determined duplexes being the one formed by oligonucleotide **44** containing three incorporations of the dihydroxylated bicyclic nucleoside **18** (T_m = 43.1 °C). Hybridization of the same modified oligonucleotide sequences **43**−**50** with complementary DNA demonstrated a general destabilization of the duplexes with decreases in melting temperatures in the range of −0.5 to −3.7 °C per modification. The destabilization is most pronounced for the sequences with three modifications and most pronounced for the incorporation of **20** (sequence **48**). On the other hand, the incorporation of **18** led to the smallest decreases in duplex stability (sequences **43** and **44**).

Circular Dichroism Spectroscopy. CD spectra for all natural and modified duplexes of the study were recorded in order to examine the duplex geometry. A- and B-type duplexes are known to display distinctly different CD spectra. A-type duplexes give an intense negative band at ~210 nm and a positive band at ~260 nm of approximately the same magnitude, where B-type duplexes give a negative band at ~250 nm and a positive band at ~275 nm. It is well-known that dsDNA duplexes adopt a B-type form in solution, whereas an RNA:RNA duplex adopts an A-type.

(35) Shaikh, K. I.; Kumar, S.; Lundhus, L.; Bond, A. D.; Sharma, P. K.; Nielsen, P. *J. Org. Chem.* **2009**, *74*, 1557–1566.

TABLE 1. Thermal Stability Data of Modified Duplexes

		T_m (ΔT_m)/°C [b]	
	ODN sequences[a]	complementary DNA 5′-dGCATATCAC-3′	complementary RNA 5′-rGCAUAUCAC-3′
42	5′-dGTGATATGC-3′	30.4	29.1
43	5′-dGTGA**X**ATGC-3′	29.9 (−0.5)	34.1 (+5.0)
44	5′-dG**X**GA**X**A**X**GC-3′	27.0 (−1.1)	43.1 (+4.7)
45	5′-dGTGA**Y**ATGC-3′	27.6 (−2.8)	32.9 (+3.8)
46	5′-dG**Y**GA**Y**A**Y**GC-3′	22.2 (−2.7)	37.5 (+2.8)
47	5′-dGTGA**Z**ATGC-3′	27.6 (−2.8)	32.3 (+3.2)
48	5′-dG**Z**GA**Z**A**Z**GC-3′	19.3 (−3.7)	35.2 (+2.1)
49	5′-dGTGA**V**ATGC-3′	28.4 (−2.0)	34.0 (+4.9)
50	5′-dG**V**GA**V**A**V**GC-3′	23.1 (−2.4)	37.9 (+3.0)

[a]Oligodeoxynucleotide sequences with **X** = **18**, **Y** = **19**, **Z** = **20**, **V** = **21** corresponding to the incorporation of **29**, **35**, **33** ,and **40**, respectively. [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, 0.1 mM EDTA, pH 7.0 using 1.5 μM concentrations of each strand. Values in parentheses show the changes in T_m values per modification compared with the reference strand.

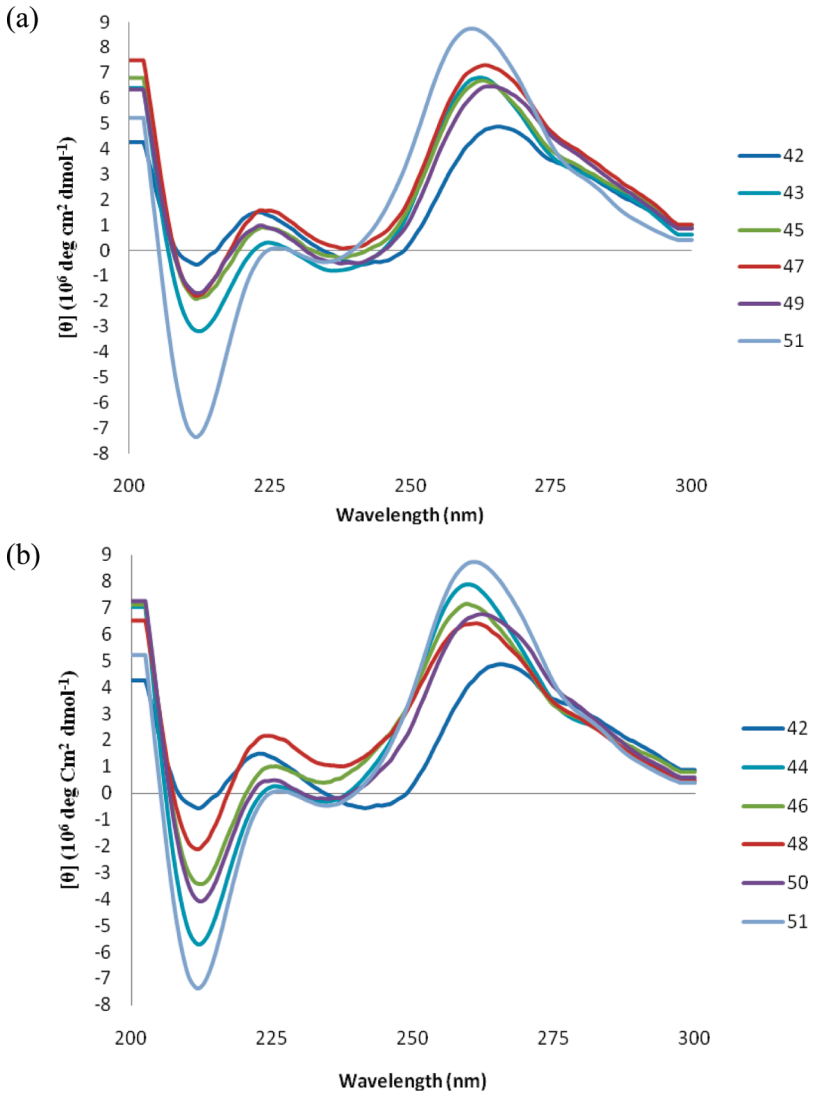


FIGURE 3. CD spectra of duplexes formed by the oligonucleotides **42**−**50** and their RNA-complements: (a) modified DNA:RNA duplexes with single bicyclic modifications; (b) modified DNA:RNA duplexes with triple bicyclic modifications. Sequence **51** corresponds to the RNA sequence 5′-rGUGAUAUGC-3′.

DNA:RNA duplexes adopt intermediate A/B-type structures. An RNA:RNA duplex is therefore taken as a standard for the A-type, and the CD-curve (see **51** in Figure 3) clearly displays the A-type characteristics with especially an intense negative band at 210 nm. This band is small for the DNA:RNA duplex (**42**). The modified DNA:RNA duplexes displays some clear A-type characteristics that are much more pronounced with three incorporations of bicyclic

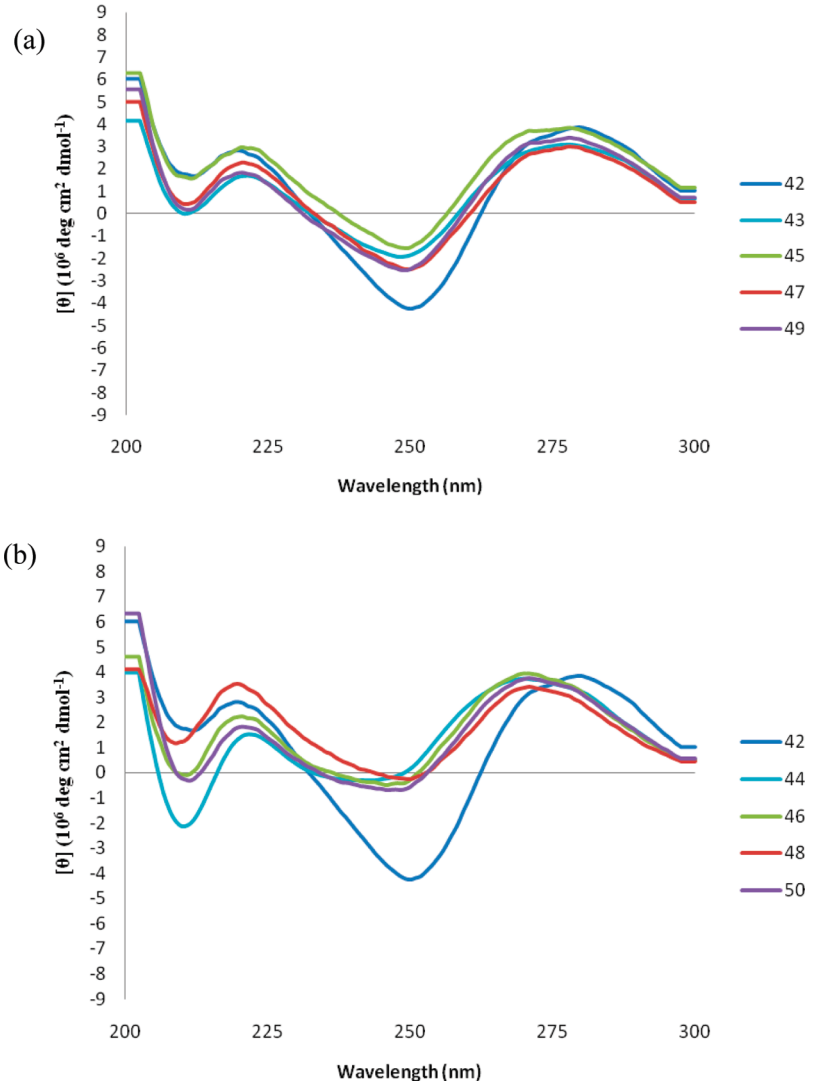


FIGURE 4. CD spectra of duplexes formed by the oligonucleotides **42**−**50** and their DNA complements: (a) modified dsDNA duplexes with single bicyclic modifications; (b) modified dsDNA duplexes with triple bicyclic modifications.

nucleosides than with single incorporations (compare parts a and b of Figure 3). The negative band at 210 nm clearly decreases in intensity in the order RNA > **18** > **21** > **19** > **20** > DNA for both one incorporation (i.e., **51** > **43** > **49** ~ **45** ~ **47** > **42**) and three incorporations (i.e., **51** > **44** > **50** > **46** > **48** > **42**) in the DNA:RNA duplex. A similar picture though less systematic is seen with the positive band at 260 nm.

The unmodified dsDNA duplex shows clear B-type characteristics (see **42** in Figure 4), whereas the modified dsDNA duplexes seem to be changed toward an intermediate duplex form as clearly indicated in the decreasing bands at 210 and 250 nm. Again the largest changes are seen with three incorporations as compared to one incorporation (compare parts a and b of Figure 4), and again, the changes are most pronounced for the modification **18** (see **44** in Figure 4b) with a negative band at 210 nm. Another trend in the CD spectra indicating a shift toward more A-type like duplexes of the modified ssDNA duplexes is that the large band at 280 nm is shifting toward 270 nm by the increasing number of modifications.

Triplex Studies. The incorporation of our native carbocyclic bicyclic nucleoside **8** into triplex-forming oligonucleotides did not lead to any siginificant increase in triplex stability,[21] and this result is in contrary to the very triplex-stabilizing effects of the 2′-oxy analogue, ENA **4**,[23] as well as of LNA **1**.[22] Therefore, it was interesting to study whether the dihydroxylated derivative **18**, which introduces hydrofilicity directly around the 2′,4′-linkage could change this picture. Hence, the 3′-*O*-phosphoramidite **29** was incorporated into the same TFO sequences as used in our former study[21] and the thermal stability data of the triplexes formed with a DNA duplex target are shown in Table 2. However, few differences in melting temperatures between the modified sequences **53**/**54** and the unmodified sequence **52** can be observed, and in general, the two hydroxy funtionalities on **18** as compared to **8** does not seem to make any significant difference on the triplex stability.

Discussion

In our previous paper, we demonstrated the efficient linear synthesis of **9** from uridine based on ring-closing metathesis as the key step.[16] Herein, we have proved that the enyne metathesis can be performed with equal efficiency to give the

TABLE 2. Thermal Stability Data of Modified Triplexes

	complementary DNA duplex:[a] 3′-GGT GAA AAA TTT TCT TTT CCC CCC TGA CC-5′ 5′-CCA CTT TTT AAAAGAAAAGGGGGGACT GG-3′	
	TFO sequences:[b]	T_m (ΔT_m)[c]/°C
52	5′-TTT TCT TTT CCC CCC T-3′	30.3
53	5′-TTT TC**X** TTT CCC CCC T-3′	29.8 (−0.5)
54	5′-TT**X** TC**X** TT**X** CCC CCC T-3′	30.1 (−0.1)

[a]DNA duplex with the target part underlined. [b]Triplex forming oligodeoxynucleotide sequences with **X** = **18** corresponding to the incorporation of **29**. [c]Melting temperatures obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in a buffer containing 10 mM sodium cacodylate, 150 mM NaCl, 10 mM $MgCl_2$, pH 6.0 using 1.0 μM concentration of the duplex and 1.5 μM concentrations of the TFO sequences. Values in parentheses show the changes in T_m values per modification compared with the reference strand.

1,3-diene-containing bicyclic nucleoside **20**. The olefins of **9** and **20** open the opportunity of preparing a range of analogues as we have proved by simple hydrogenation to give **8** and **19** as well as the hydroxylated analogues **18** and **21** (or at first, protected forms thereof). Finally, the potential of **20** for Diels−Alder reactions has been enlightened by the preparation of **41**. Clearly, a much larger variety of bi- and tricyclic nucleosides could be designed by using the two key building blocks **9** and **20** in order to explore the effects of decoration of the 2′,4′-linkage and tuning the electrostatics in the nucleic acid structure as well as for the conjugation of the oligonucleotides to other entities. The application of Diels−Alder reactions on oligonucleotides containing **20** will be applied in future studies.

The thermal stability data of the present study continues the line in the growing series of different bicyclic nucleoside building blocks with 2′−4′-linkages. When comparing the hybridization properties of LNA and ENA with their 2′-carba analogues **13** (although 7′-methylated) and **8**, respectively, the presence of a 2′-oxygen is clearly of high importance, most probably due to hydration. The feature behind the ability of LNA−DNA mixmers to form the stabilized A-type duplexes with complementary DNA and RNA has been suggested to be the conformational steering of the neighboring 2′-deoxynucleotides toward *N*-type puckering,[7] and this effect seems related to this hydration. Herein, we have extended this study by attaching hydrophilic/hydrophobic groups to **8** and **9** from our former study,[16] in order to see whether only the 2′-oxygen can induce the hydration or whether this can be obtained by other placements of an oxygen or negatively influenced by hydrophobic groups. Concerning the DNA:RNA duplexes of this study, the two entirely hydrophobic bicyclic nucleosides **19** (as an 8:1 epimeric mixture), and **20** did, in fact, lead to the smallest increases in duplex stability at the same range though slightly smaller than their 6′-unsubstituted analogues **8** and **9**. Like with **8** and **9**, the saturated analogue **19** leads to slightly higher T_m's than the unsaturated **20**. The introduction of hydrophilic hydroxyl groups improves the RNA-affinity with **21** being superior to **20** and **9**, and with **18** being superior to **19** and **8**. Especially, the melting temperature of the DNA:RNA duplex formed with the triple modified sequence **44** is high, 43 °C, as compared to 38 °C with **8** instead of **18**.[16] These trends are clearly related to the CD spectra (Figure 3), showing that the shift in duplex form toward a more A-type like structure is largest for **18** decreasing in the order **18** > **21** > **19** > **20**. This is coherent with the former study showing larger shifts for the saturated **8** as compared to the unsaturated **9** but also lower shifts for both as compared to LNA.[16] Hence, the steering performed on neighboring 2′-deoxynucleotides seems from the CD spectra to be less pronounced for our carbocyclic analogues as compared to LNA. This confirms the trend that the more conformational steering toward A-type duplex formation, the higher is the thermal stability. That this steering is directly related to the 2′-oxygen atom is confirmed by ENA **4** as well as **7** being more or less as efficient as LNA **1** in hybridizing to RNA, whereas all carbocyclic analogues show slightly lower RNA-affinities. A partly compensation for the lack of 2′-oxygen can be obtained by other hydrophilic groups in the bridge as seen for **18** and to some extent for **21**. This indicates again that water binding in the minor groove has a large influence on the duplex formation, but it should be noted that the 2′-oxygen is positioned deeply into the minor groove, whereas the 6′/7′-substituents are positioned more at the rim of the groove. Probably due to steric reasons, all the 8′-methylated analogues **16**−**17** generally demonstrate lower RNA-affinity than the 8′-unsubstituted analogues of this study.[16,20] However, it should be addressed that different sequence contexts hamper direct comparison. Also in the 8′-methylated series, however, the introduction of a 6′-hydroxyl group improves the RNA-affinity.[20]

In the formation of DNA:DNA duplexes, the indicated effects of water binding are even more pronounced. Wheras LNA, and to a large extent ENA, reveals strong duplex stabilization, this decreases dramatically with other analogues. The new analogues shown herein all lead to decreased duplex stability when incorporated one or three times, with the decrease being more pronounced for the hydrophobic analogues **19** and **20** than for the hydroxylated **18** and **21**. The hydrophobic substituents of **19** and **20** seem to decrease the duplex stability even further as compared to **8** and **9**.[16] The conformational steering as indicated by CD-spectroscopy (Figure 4) follows the trend. Thus, the presence of a 2′-oxygen is crucial for the steering and therefore for the duplex stability, and the compensation by other hydrophilic groups is only small. Increases in DNA:DNA duplex stability by this series of locked nucleic acid analogues has in general only been seen with 2′-oxygens like in LNA **1**, ENA **4**, and **7** though with native LNA being superior, and the effect also found with **2** and **3** indicating that the smaller ring (and higher puckering) is also playing a role. Also the 7′/8′-methylated carbocyclic analogues **13**−**14** and **15**−**17** are showing decreased DNA:DNA duplex stability, although some compensation by a 6′-hydroxyl group is seen for one stereoisomer of **13**.[20] The larger effect of hydration in the modified DNA:DNA duplexes as compared to the modified DNA:RNA duplexes can be related to the more extended structure and thereby larger surface of a B-type or B-type like duplex than for an A-type or A-type-like duplex.

In the formation of triplexes, the same trend is seen, as both LNA **1**, amino-LNA **3**, the longer ENA **4**, and **7** form very stable triplexes, whereas our carbocyclic analogues **8** and its hydroxylated analogue **18** do not lead to significant increases in stability as compared to the native oligonucleotides. In other words, the 2′-heteroatom is crucial, and compensation has not been found with the two hydroxyl groups at the ring, although negative sterical influence by the additional groups cannot be excluded. This is in full accordance with an NMR study indicating that triplexes formed by LNA have a special structure with a more efficient network of hydration.[36] Apparently, this hydration is related directly to the 2′-heteroatom, as ENA and **7** give highly stable triplexes, whereas the carbocyclic analogues of ENA do not.

In summary, the nucleic acid recognition performed by the carbocyclic locked nucleic acid analogues of the present study is very RNA-selective. Only with RNA and not with single- or double-stranded DNA has a general increase in thermal stability been found. This might be a useful feature for diagnostic applications that are not found for the native LNA and other analogues.

Conclusion

We have synthesized four different modified nucleosides starting from uridine by ring-closing diene or enyne metathesis. All nucleosides were successfully incorporated into oligonucleotides, and the hybridization properties of these were recorded. The modified nucleosides have shown an increase in melting temperatures of +2.1 to +5.0 °C per modification against RNA, a decrease of −0.5 to −3.7 °C per modification against DNA, and very small influence on triplex formation. This gives new information on the importance of the 2′-oxygen atom for the formation of duplexes and triplexes. Some compensation for the lack of a 2′-oxygen atom in an all carbocyclic additional ring can be obtained with other hydrophilic groups attached to the ring. Importantly, the perspective of using the 1,3-diene of **20** for further conjugation using the Diels−Alder reaction has been demonstrated.

Experimental Section

Synthesis of (1*R*,2*S*,3*S*,5*R*,6*R*,8*S*)-2,3-Dihydroxy-8-*tert*-butyldimethylsilyloxy-1-*tert*-butyldimethylsilyloxymethyl-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]octane (25). Nucleoside **24**[16] (509 mg, 1.03 mmol) was dissolved in THF (9 mL) and H_2O (9 mL) in a microwave vial. *N*-methylmorpholine *N*-oxide (362 mg, 3.09 mmol) and a 2.5% w/w solution of OsO_4 in *tert*-butyl alcohol (519 μL, 0.051 mmol) were added, and the solution was stirred in the microwave reactor at 100 °C for 20 min. A 5% aqueous solution of $Na_2S_2O_5$ (7 mL) was added, and the solution was concentrated under reduced pressure to approximately 15 mL. The solution was extracted with EtOAc (4 × 50 mL), and the combined organic extracts were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc−petroleum ether, 1:1 v/v) to give the desired product **25** (429 mg, 79%) as a white foam: R_f 0.60 (EtOAc); ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.29 (br s, 1H, NH), 8.15 (d, 1H, J = 8.0 Hz, H-6), 6.04 (s, 1H, H-1′), 5.39 (dd, 1H, J = 1.6, 8.0 Hz, H-5), 4.63 (d, 1H, J = 2.8 Hz, 7′-OH), 4.60 (d, 1H, J = 8.7 Hz, 6′-OH), 4.35 (d, 1H, J = 5.2 Hz, H-3′), 4.15, 3.55 (AB, 2H, J = 11.8 Hz, H-5′), 3.92 (m, 1H, H-7′), 3.62 (dd, 1H, J = 5.4, 8.7 Hz, H-6′), 2.30 (m, 1H, H-2′), 2.01 (m, 1H, H-8′$_b$), 1.84 (m, 1H, H-8′$_a$), 0.92 (s, 9H, SiC(CH$_3$)$_3$), 0.88 (s, 9H, SiC(CH$_3$)$_3$), 0.11 (s, 3H, SiCH$_3$), 0.05 (s, 3H, SiCH$_3$) 0.05 (s, 3H, SiCH$_3$), 0.02 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8 (C-4), 150.6 (C-2), 140.7 (C-6), 100.9 (C-5), 87.3, 87.1 (C-1′, C-4′), 68.5 (C-6′), 67.1 (C-3′), 66.7 (C-7′), 61.0 (C-5′), 44.2 (C-2′), 28.4 (C-8′), 26.1, 25.6 (SiC(*C*H$_3$)$_3$), 18.5, 17.8 (Si*C*(CH$_3$)$_3$), −4.7, −5.2, −5.3, −5.6 (SiCH$_3$); MALDI MS m/z (551.2557 [M + Na]$^+$, $C_{24}H_{44}N_2O_7Si_2$−Na$^+$ calcd 551.2579).

Synthesis of (1*R*,2*S*,3*S*,5*R*,6*R*,8*S*)-2,3-Diacetyloxy-8-*tert*-butyldimethylsilyloxy-1-*tert*-butyldimethylsilyloxymethyl-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]octane (26). Nucleoside **25** (850 mg, 1.609 mmol) was coevaporated with anhydrous pyridine (4 mL) and redissolved in the same solvent (14 mL). The solution was stirred at 0 °C, and DMAP (59 mg, 0.483 mmol) and acetic anhydride (334 μL, 3.54 mmol) were added. The solution was stirred at 0 °C for 15 min and at room temperature for 1.5 h. Additional acetic anhydride (152 μL, 1.61 mmol) was added, and after 1 h, a similar portion was added. The solution was stirred for 19 h, and a saturated aqueous solution of $NaHCO_3$ (20 mL), and CH_2Cl_2 (100 mL) was added. The residue was extracted with CH_2Cl_2 (3 × 125 mL) and the combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$ (100 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc−petroleum ether, 1:3 v/v) to give the desired product **26** (920 mg, 93%) as a white foam: R_f 0.60 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.08 (br s, 1H, NH), 8.35 (d, 1H, J = 8.1 Hz, H-6), 6.08 (s, 1H, H-1′), 5.67 (d, 1H, J = 8.1 Hz, H-5), 5.50 (m, 1H, H-7′), 5.31 (d, 1H, J = 5.7 Hz, H-6′), 4.48 (d, 1H, J = 5.1 Hz, H-3′), 4.00, 3.52 (AB, 2H, J = 11.4 Hz, H-5′), 2.44 (m, 1H, H-2′), 2.35 (m, 1H, H-8′), 2.13 (s, 3H, CH_3CO), 2.09−2.03 (m, 4H, H-8′, CH_3CO), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.91 (s, 9H, SiC(CH$_3$)$_3$), 0.13 (s, 3H, SiCH$_3$), 0.12 (s, 3H, SiCH$_3$) 0.09 (s, 3H, SiCH$_3$), 0.07 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 169.7 (CO), 163.5 (C-4), 150.2 (C-2), 140.3 (C-6), 101.0 (C-5), 87.4, 86.0 (C-1′, C-4′), 68.8 (C-6′), 67.2 (C-3′), 66.5 (C-7′), 59.8 (C-5′), 44.1 (C-2′), 26.3 (C-8′), 26.0, 25.6 (SiC(*C*H$_3$)$_3$), 21.2, 20.5 (*C*H$_3$CO), 18.5, 17.8 (Si*C*(CH$_3$)$_3$), −4.6, −5.2, −5.3, −5.6 (SiCH$_3$); ESI MS m/z (635.2799 [M + Na]$^+$, $C_{28}H_{48}N_2O_9Si_2$−Na$^+$ calcd 635.2791).

Synthesis of (1*R*,2*R*,3*S*,5*R*,6*R*,8*S*)-2,3-Diacetyloxy-8-hydroxy-1-(4,4′-dimethoxytrityloxymethyl)-6-(uracil-1-yl)-7-oxabicyclo-[3.2.1]octane (28). Nucleoside **26** (878 mg, 1.43 mmol) was dissolved in anhydrous THF (30 mL), and a 1 M solution of TBAF in anhydrous THF (3.15 mL, 3.15 mmol) was added. The mixture was stirred at room temperature for 30 min and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (EtOAc−petroleum ether, 9:1 v/v) to give the crude desired product **27** (230 mg, 42%) as a white foam, which was used without further purification in the next step (R_f 0.10 (EtOAc); ESI MS m/z 407.1069 [M + Na]$^+$, $C_{16}H_{20}N_2O_9$−Na$^+$ calcd 407.1061), as well as a mixture of deacetylated products (510 mg). Nucleoside **27** (220 mg, 0.572 mmol) was coevaporated with anhydrous pyridine (2 mL) and redissolved in a mixture of the same solvent (2.5 mL) and anhydrous CH_3CN (2.5 mL). DMT-Cl (194 mg, 0.572 mmol) was added and the reaction mixture was stirred at room temperature for 22 h. An additional amount of DMT-Cl (39 mg, 0.114 mmol) was added, and after stirring for 5 h the mixture was concetrated under reduced pressure. The residue was purified by silica gel column chromatography (0−1% CH_3OH and 0.5% pyridine in CH_2Cl_2) to give the desired product **28** (310 mg, 79%) as a white foam: R_f 0.30 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 1H, NH), 8.09 (d, 1H, J = 8.1 Hz, H-6), 7.40−7.25 (m, 9H, Ar), 6.84 (dd, 4H, J = 1.2, 8.7 Hz, Ar), 6.04 (s, 1H, H-1′), 5.47 (t, 1H, J = 5.3 Hz, H-7′), 5.40 (d, 1H, J = 8.1 Hz, H-5), 5.34 (d, 1H, J = 5.3 Hz, H-6′),

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4.42 (m, 1H, H-3′), 3.80 (s, 6H, OCH_3), 3.60, 3.45 (AB, 2H, J = 10.8 Hz, H-5′), 2.80 (d, 1H, J = 3.0 Hz, OH), 2.55 (m, 1H, H-2′), 2.43 (m, 1H, H-8′), 2.09 (s, 3H, CH_3CO), 2.02 (m, 1H, H-8′), 1.88 (s, 3H, CH_3CO); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3, 169.5-(CO), 163.6 (C-4), 158.8, 158.8 (Ar), 150.2 (C-2), 144.0 (Ar), 140.2 (C-6), 136.2, 130.1, 130.0, 128.1, 128.0, 127.3, 113.4 (Ar), 101.1 (C-5), 87.8, 87.6 (C-1′, C-4′), 84.9 (*C*Ar$_3$), 68.6 (C-6′), 68.4 (C-3′), 66.7 (C-7′), 60.7 (C-5′), 55.2 (OCH_3), 43.9 (C-2′), 25.8 (C-8′), 21.2, 20.3 (*C*H_3CO); ESI MS m/z (709.2176 [M + Na]$^+$, $C_{37}H_{38}N_2O_{11}$−Na$^+$ calcd 709.2368).

Synthesis of (1*R*,2*S*,3*S*,5*R*,6*R*,8*S*)-2,3-Diacetyloxy-8-cyanoethoxy(diisopropylamino)phosphinoxy-1-(4,4′-dimethoxytrityloxymethyl)-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]octane (29). A solution of nucleoside **28** (134 mg, 0.195 mmol) in anhydrous DCE (2.5 mL) was stirred at roon temperature. *N,N*-Diisopropylethylamine (170 μL, 0.977 mmol) and *N,N*-diisopropylamino-2-cyanoethylphosphinochloridite (131 μL, 0.587 mmol) were added, and the reaction mixture was stirred at room temperature for 24 h. CH_2Cl_2 (15 mL) was added, and the mixture was washed with a saturated aqueous solution of $NaHCO_3$ (15 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 15 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−0.5% CH_3OH and 1% pyridine in CH_2Cl_2) to give the desired product **29** (173 mg, 100%) as a white foam: R_f 0.60 (EtOAc); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 151.6, 149.5; ESI MS m/z (909.3404 [M + Na]$^+$, $C_{46}H_{55}N_4O_{12}P$−Na$^+$ calcd 909.3446).

Synthesis of 2′-*C*-Allyl-2′-deoxy-3′,5′-di-*O*-(*tert*-butyldimethylsilyl)-4′-*C*-ethynyluridine (30). To a stirred solution of nucleoside **22** (2.186 g, 4.16 mmol) in anhydrous CH_2Cl_2 (35 mL) was added Dess−Martin periodinane (2.203 g, 5.19 mmol). The mixture was stirred at room temperature for 2 h and then filtered through Celite. The filter was washed with EtOAc (30 mL), and the combined organic phases were washed with a mixture of saturated aqueous solutions of $Na_2S_2O_3$ and $NaHCO_3$ (1:1, v/v, 40 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure to give the crude aldehyde (2.301 g). A suspension of K_2CO_3 (2.871 g, 20.78 mmol) and *p*-toluenesulfonyl azide (2.048 g, 10.39 mmol) in anhydrous CH_3CN (10 mL) was stirred at room temperature, and dimethyl-2-oxopropylphosphonate (1.42 mL, 10.39 mmol) was added. The mixture was stirred for 2 h, and a solution of the aldehyde (2.301 g, 4.16 mmol) in anhydrous CH_3OH (10 mL) was added. The mixture was stirred for 24 h and concentrated under reduced pressure. The residue was dissolved in Et_2O (20 mL) and H_2O (12 mL). The aqueous layer was separated and the organic layer washed with H_2O (12 mL) and brine (12 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc−petroleum ether, 1:4 v/v) to give **30** (1.394 g, 65%) as a white foam: R_f 0.70 (EtOAc−petroleum ether, 1:1 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85 (br s, 1H, NH), 7.63 (d, 1H, J = 8.4 Hz, H-6), 6.18 (d, 1H, J = 6.6 Hz, H-1′), 5.72−5.64 (m, 2H, H-5, C*H*=CH$_2$), 5.08−4.96 (m, 2H, CH=C*H*$_2$), 4.39 (d, 1H, J = 6.0 Hz, H-3′), 3.90, 3.76 (AB, 2H, J = 11.1 Hz, H-5′), 2.56 (s, 1H, HC≡C), 2.46−2.32 (m, 3H, H-2′, C*H*$_2$CH=CH$_2$), 0.96 (s, 9H, SiC(CH$_3$)$_3$), 0.94 (s, 9H, SiC(CH$_3$)$_3$), 0.13 (s, 3H, SiCH$_3$), 0.13 (s, 3H, SiCH$_3$), 0.12 (s, 3H, SiCH$_3$), 0.09 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.0 (C-4), 150.1 (C-2), 140.3 (C-6), 135.3 (*C*H=CH$_2$), 116.9 (CH=*C*H$_2$), 102.8 (C-5), 88.1 (C-1′), 85.0, 80.5, 77.6 (C-4′, C≡CH), 73.7 (C-3′), 67.1 (C-5′), 49.3 (C-2′), 30.1 (*C*H$_2$CH=CH$_2$), 26.0, 25.9 (SiC(*C*H$_3$)$_3$), 18.4, 18.3 (Si*C*(CH$_3$)$_3$), −3.9, −4.1, −5.3, −5.3 (SiCH$_3$); HRMALDI MS m/z (543.2684 [M + Na]$^+$, $C_{26}H_{44}N_2O_5Si_2$−Na$^+$ calcd 543.2681).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-(*tert*-butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)-6-(uracil-1-yl)-2-vinyl-7-oxabicyclo[3.2.1]oct-2-ene (31). To a stirred solution of **30** (1.389 g, 2.67 mmol) in anhydrous CH_2Cl_2 (6 mL) was added Grubbs' second-generation catalyst (((Mes)$_2$Im)(Cy$_3$P)Cl$_2$Ru=CHPh) (113 mg, 0.13 mmol). The solution was stirred in a microwave reactor at 100 °C for 2 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc−petroleum ether, 1:4 v/v) to give the bicyclic nucleoside **31** (1.134 g, 82%) as a white foam: R_f 0.70 (EtOAc−petroleum ether, 1:1 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.09 (br s, 1H, NH), 8.16 (d, 1H, J = 8.1 Hz, H-6), 6.09 (m, 1H, C*H* = CH$_2$), 5.88 (m, 1H, H-7′), 5.68−5.61 (m, 2H, H-5, H-1′), 5.28 (dd, 1H, J = 1.8, 16.5 Hz, CH=C*H*$_2$), 5.0 (dd, 1H, J = 1.8, 10.5 Hz, CH=C*H*$_2$), 4.46 (d, 1H, J = 5.1 Hz, H-3′), 4.04, 3.65 (AB, 2H, J = 11.4 Hz, H-5′), 2.62 (m, 1H, H-8′), 2.40−2.33 (m, 2H, H-2′, H-8′), 0.95 (s, 9H, SiC(CH$_3$)$_3$), 0.84 (s, 9H, SiC(CH$_3$)$_3$), 0.14 (s, 3H, SiCH$_3$), 0.12 (s, 3H, SiCH$_3$), 0.06 (s, 3H, SiCH$_3$), 0.05 (s, 3H, SiCH$_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7 (C-4), 150.3 (C-2), 140.3 (C-6), 138.5 (C-6′), 133.1 (*C*H=CH$_2$), 125.7 (C-7′), 116.6 (CH=*C*H$_2$), 101.2 (C-5), 89.2 (C-1′), 83.4 (C-4′), 65.2 (C-3′), 60.6 (C-5′), 44.6 (C-2′), 28.2 (C-8′), 26.1, 25.9 (SiC(*C*H$_3$)$_3$), 18.5, 17.9 (Si*C*(CH$_3$)$_3$), −4.4, −4.9, −5.2, −5.3 (SiCH$_3$); HRMALDI MS m/z (543.2659 [M + Na]$^+$ $C_{26}H_{44}N_2O_5Si_2$−Na$^+$ calcd 543.2681).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-hydroxy-1-hydroxymethyl-6-(uracil-1-yl)-2-vinyl-7-oxabicyclo[3.2.1]oct-2-ene (20). A solution of nucleoside **31** (529 mg, 1.02 mmol) in anhydrous CH_3CN (10 mL) was added KF (0.886 g, 15.25 mmol) and 18-crown ether-6 (1.075 g, 4.07 mmol). The solution was stirred in a microwave reactor at 100 °C for 1 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (0−5% CH_3OH in CH_2Cl_2) to give **20** (212 mg, 71%) as a white foam: R_f 0.40 (MeOH-dichloromethane, 1:9 v/v); ^{1}H NMR (300 MHz, CD_3OD) δ 8.27 (d, 1H, J = 8.1 Hz, H-6), 6.19 (dd, 1H, J = 10.8, 17.1 Hz, C*H* = CH$_2$), 5.95 (m, 1H, H-7′), 5.65 (d, 1H, J = 8.1 Hz, H-5), 5.57 (s, 1H, H-1′), 5.34 (dd, 1H, J = 2.1, 17.1 Hz, CH=C*H*$_2$), 5.01 (dd, 1H, J = 2.1, 10.8 Hz, CH=C*H*$_2$), 4.50 (d, 1H, J = 5.4 Hz, H-3′), 4.03, 3.72 (AB, 2H, J = 12.0 Hz, H-5′), 2.67 (m, 1H, H-8′), 2.45 (m, 1H, H-2′), 2.33 (m, 1H, H-8′); ^{13}C NMR (75 MHz, CD_3OD) δ 166.5 (C-4), 152.1 (C-2), 142.2 (C-6), 139.8 (C-6′), 134.6 (*C*H=CH$_2$), 126.9 (C-7′), 116.3 (CH=*C*H$_2$), 101.3 (C-5), 90.5 (C-1′), 84.2 (C-4′), 65.9 (C-3′), 60.3 (C-5′), 45.2 (C-2′), 28.7 (C-8′); HRMALDI MS m/z (315.0960 [M + Na]$^+$, $C_{14}H_{16}N_2O_5$−Na$^+$ calcd 315.0951).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-1-(4,4′-Dimethoxytrityoxymethyl)-8-hydroxy-6-(uracil-1-yl)-2-vinyl-7-oxabicyclo[3.2.1]oct-2-ene (32). DMT-Cl (79 mg, 0.23 mmol) was added to a stirred solution of **20** (34 mg, 0.12 mmol) in anhydrous pyridine (0.75 mL) and anhydrous CH_3CN (0.75 mL). The mixture was stirred for 22 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−3.0% CH_3OH and 0.5% pyridine in CH_2Cl_2) to give the product **32** (45 mg, 65%) as a foam: R_f 0.70 (CH_3OH−dichloromethane, 1:9 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.17 (br s, 1H, NH), 8.19 (d, 1H, J = 8.1 Hz, H-6), 7.46−7.14 (m, 9H, Ar), 6.87 (dd, 4H, J = 1.8, 8.7 Hz, Ar), 6.01 (m, 1H, H-7′), 5.88 (dd, 1H, J = 10.8, 17.1 Hz, C*H*=CH$_2$), 5.66 (s, 1H, H-1′), 5.41 (d, 1H, J = 8.1 Hz, H-5), 5.29 (dd, 1H, J = 1.5, 17.1 Hz, CH=C*H*$_2$), 4.95 (dd, 1H, J = 1.5, 10.8 Hz, CH=C*H*$_2$), 4.69 (t, 1H, J = 6.3 Hz, H-3′), 3.80 (s, 6H, OCH_3), 3.71, 3.46 (AB, 2H, J = 11.1 Hz, H-5′), 2.63−2.48 (m, 3H, H-8′, H-2′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.3 (C-4), 158.8 (Ar), 150.3 (C-2), 144.5 (Ar), 140.3 (C-6), 138.3, 135.3, 135.2 (C-6′, Ar), 132.4 (*C*H=CH$_2$), 130.3, 130.2, 129.1, 128.3, 128.2, 127.3 (Ar), 126.1 (C-7′), 117.0 (CH=*C*H$_2$), 113.5 (Ar), 101.4 (C-5), 89.4 (C-1′), 87.4, 82.4 (CAr$_3$, C-4′), 67.0 (C-3′), 61.1 (C-5′), 55.3 (OCH_3), 43.8 (C-2′), 27.5 (C-8′); HRMALDI MS m/z (617.2264 [M + Na]$^+$, $C_{35}H_{34}N_2O_7$−Na$^+$ calcd 617.2258).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-6-(uracil-1-yl)-2-vinyl-7-oxabicyclo[3.2.1]oct-2-ene (33). To a stirred solution of nucleoside **32** (50 mg, 0.08 mmol) in anhydrous CH_2Cl_2 (1 mL) were added *N*,*N*-diisopropylethylamine (73 μL, 0.42 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (56 μL, 0.25 mmol). The mixture was stirred for 21 h and CH_2Cl_2 (2 mL) was added. The mixture was washed with a saturated aqueous solution of $NaHCO_3$ (2 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 3 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−2% CH_3OH and 0.5% pyridine in CH_2Cl_2) to give **33** (40 mg, 60%) as a white foam: R_f 0.80 (CH_3OH−dichloromethane, 1:9 v/v); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 149.7, 149.4; ESI MS *m*/*z* (817.3331 $[M + Na]^+$, $C_{44}H_{51}N_4O_8P-Na^+$ calcd 817.3337).

Synthesis of 2(*R*/*S*)-(1*R*,5*R*,6*R*,8*S*)-2-Ethyl-8-hydroxy-1-(hydroxymethyl)-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]octane (19). To a stirred solution of bicyclic nucleoside **20** (0.128 g, 0.44 mmol) in CH_3OH (3 mL) was added PtO_2 (0.053 g, 0.23 mmol), and the mixture was stirred under hydrogen atmosphere for 24 h. The mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−6% CH_3OH in CH_2Cl_2) to give **19** (as a mixture of diastereomers in 8:1 ratio, 90 mg, 70%) as a white foam: R_f 0.40 (CH_3OH−dichloromethane, 1:9 v/v); ^1H NMR (300 MHz, CD_3OD) δ 8.55 (d, 1H, *J* = 8.1 Hz, H-6), 8.44 (d, 1H, *J* = 8.1 Hz, H-6), 5.72 (s, 1H, H-1′), 5.68 (s, 1H, H-1′), 5.62 (d, 1H, *J* = 8.1 Hz, H-5), 5.60 (d, 1H, *J* = 8.1 Hz, H-5), 4.50 (d, 1H, *J* = 5.7 Hz, H-3′), 4.37 (d, 1H, *J* = 5.1 Hz, H-3′), 3.95, 3.58 (AB, 2H, *J* = 11.7 Hz, H-5′), 3.83, 3.51 (AB, 2H, *J* = 12.0 Hz, H-5′), 2.34 (br s, 2H, 2 × H-2′), 2.08−1.14 (m, H-6′, H-7′, H-8′, CH_3CH_2), 0.89−0.84 (m, 6H, 2 × CH_3); ^{13}C NMR (major isomer) (75 MHz, CD_3OD) δ 166.4 (C-4), 150.9 (C-2), 141.6 (C-6), 99.4 (C-5), 88.2, 87.7 (C-1′, C-4′), 64.9 (C-3′), 60.2 (C-5′), 45.2 (C-2′), 36.2, 23.6, 21.8, 19.8 (C-6′, C-7′, C-8′, CH_3CH_2), 10.4 (CH_3); HRMALDI MS *m*/*z* (319.1271 $[M + Na]^+$, $C_{14}H_{20}N_2O_5-Na^+$ calcd 319.1265).

Synthesis of 2(*R*/*S*)-(1*R*,5*R*,6*R*,8*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-2-ethyl-8-hydroxy-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]-octane (34). A solution of **19** (0.090 g, 0.30 mmol) in anhydrous pyridine (1.5 mL) and anhydrous CH_3CN (1.5 mL) was stirred at room temperature and DMT-Cl (0.206 g, 0.61 mmol) was added. The mixture was stirred for 23 h and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−2.5% CH_3OH and 0.5% pyridine in CH_2Cl_2) to give **34** (as a mixture of diastereomers in a 8:1 ratio, 130 mg, 72%) as a foam: R_f 0.70 (CH_3OH−dichloromethane, 1:9 v/v); ^1H NMR (300 MHz, $CDCl_3$) δ 9.07 (br s, 2H, 2 × NH), 8.29 (d, 1H, *J* = 8.1 Hz, H-6), 8.17 (d, 1H, *J* = 8.1 Hz, H-6), 7.46−7.15 (m, 18H, Ar), 6.88−6.82 (m, 8H, Ar), 5.78 (s, 1H, H-1′), 5.73 (s, 1H, H-1′), 5.32−5.23 (m, 2H, H-5), 4.52 (br s, 1H, H-3′), 4.14 (br s, 1H, H-3′), 3.78 (s, 12H, OCH_3), 3.60, 3.34 (AB, 2H, *J* = 10.8 Hz, H-5′), 3.48, 3.39 (AB, 2H, *J* = 11.7 Hz, H-5′), 2.45−2.40 (m, H-2′), 1.99−0.95 (m, H-6′, H-7′, H-8′, CH_3CH_2), 0.87−0.72 (m, CH_3); ^{13}C NMR (major isomer) (75 MHz, $CDCl_3$) δ 163.7 (C-4), 158.8 (Ar), 150.3 (C-2), 144.5 (Ar), 140.9 (C-6), 135.6, 135.3, 130.2, 128.3, 128.2, 127.3, 127.2, 113.4 (Ar), 101.0 (C-5), 87.7, 87.5, 87.4 (C-1′, C-4′, CAr_3), 67.4 (C-3′), 62.5 (C-5′), 55.4 (OCH_3), 45.1 (C-2′), 36.6, 23.5, 21.9, 19.9 (C-6′, C-7′, C-8′, CH_3CH_2), 11.2 (CH_3); HRMALDI MS *m*/*z* (621.2587 $[M + Na]^+$, $C_{35}H_{38}N_2O_7-Na^+$ calcd 621.2572).

Synthesis of 2(*R*/*S*)-(1*R*,5*R*,6*R*,8*S*)-8-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-2-ethyl-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]octane (35). To a stirred solution of compound **34** (125 mg, 0.21 mmol) in anhydrous CH_2Cl_2 (1.5 mL) were added *N*,*N*-diisopropylethylamine (0.22 mL, 1.25 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.14 mL, 0.63 mmol). The mixture was stirred for 15 h, and then CH_2Cl_2 (2 mL) was added. The mixture was washed with a saturated aqueous solution of $NaHCO_3$ (2 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 3 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−1.5% CH_3OH and 0.5% pyridine in CH_2Cl_2) to give **35** (80 mg, 48%) as a white foam: R_f 0.80 (CH_3OH−-dichloromethane, 1:9 v/v); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 150.2 (major isomer), 150.0 (minor isomer), 149.9 (minor isomer), 149.5 (major isomer); ESI MS *m*/*z* (821.3659 $[M + Na]^+$, $C_{44}H_{55}N_4O_8P-Na^+$ calcd 821.3650).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-8-(*tert*-Butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)-2-hydroxymethyl-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]oct-2-ene (36). A solution of nucleoside **31** (675 mg, 1.30 mmol) in *t*-BuOH (20 mL) was added to a solution of K_3FeCN_6 (1.282 g, 3.89 mmol), K_2CO_3 (537 mg, 3.89 mmol), $K_2OsO_2(OH)_4$ (10 mg, 0.03 mmol), and $(DHQ)_2$-PHAL (101 mg, 0.13 mmol) in water (15 mL), and the resulting mixture was stirred at room temperature for 4 h. Solid Na_2SO_3 (1.4 g, 11.1 mmol) was added, and the mixture was stirred for 30 min. Et_2O (20 mL) was added, and the layers were separated. The aqueous layer was extracted with Et_2O (2 × 5 mL), and the combined organic layers were washed with brine (2 × 8 mL) and dried (Na_2SO_4). The mixture was concentrated under reduced pressure, and the residue was dissolved in a mixture of THF and H_2O (1:1, 20 mL, v/v). $NaIO_4$ (833 mg, 3.89 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. Et_2O (10 mL) was added, and the layers were separated. The aqueous layer was extracted with Et_2O (2 × 8 mL), and the combined organic layers were washed with brine (2 × 10 mL) and dried (Na_2SO_4). The mixture was concentrated under reduced pressure to give the crude aldehyde (910 mg), which was dissolved in CH_3OH (5 mL). A 0.4 M solution of $CeCl_3 \cdot 7H_2O$ in CH_3OH (3.24 mL, 1.30 mmol) and then $NaBH_4$ (0.049 g, 1.29 mmol) were added, and the reaction mixture was stirred at room temperature for 5 min. H_2O (2 mL) was added, and the mixture was extracted in Et_2O (2 × 3 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−4% CH_3OH in CH_2Cl_2) to give **36** (368 mg, 54%) as a white foam: R_f 0.60 (CH_3OH−dichloromethane, 1:9 v/v); ^1H NMR (300 MHz, $CDCl_3$) δ 8.93 (br s, 1H, NH), 8.17 (d, 1H, *J* = 8.1 Hz, H-6), 5.86 (m, 1H, H-7′), 5.69−5.62 (m, 2H, H-5, H-1′), 4.45 (d, 1H, *J* = 5.7 Hz, H-3′), 4.29, 3.88 (AB, 2H, *J* = 11.4 Hz, H-5′), 4.03 (m, 2H, CH_2OH), 2.58−2.52 (m, 1H, H-8′), 2.37−2.30 (m, 2H, H-8′, H-2′), 0.95 (s, 9H, $SiC(CH_3)_3$), 0.84 (s, 9H, $SiC(CH_3)_3$), 0.14 (s, 3H, $SiCH_3$), 0.13 (s, 3H, $SiCH_3$), 0.07 (s, 3H, $SiCH_3$), 0.05 (s, 3H, $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.5 (C-4), 150.2 (C-2), 140.4 (C-6), 138.0 (C-6′), 129.1 (C-7′), 101.2 (C-5), 89.2 (C-1′), 83.5 (C-4′), 65.5 (C-3′), 64.0 (CH_2OH), 59.8 (C-5′), 44.5 (C-2′), 27.9 (C-8′), 26.1, 25.7 ($SiC(CH_3)_3$), 18.5, 18.0 ($SiC(CH_3)_3$), −4.5, −4.8, −5.1, −5.3 ($SiCH_3$); HRMALDI MS *m*/*z* (547.2621 $[M + Na]^+$, $C_{25}H_{44}N_2O_6Si_2-Na^+$ calcd 547.2630).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-2-Benzoyloxymethyl-8-(*tert*-butyldimethylsilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]oct-2-ene (37). Compound **36** (368 mg, 0.70 mmol) was dissolved in anhydrous pyridine (3 mL), and the resulting mixture was stirred at 0 °C. Benzoyl chloride (0.114 mL, 0.98 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under pressure, and the residue was dissolved in EtOAc (5 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (2 mL). The aqueous phase was extracted with EtOAc (2 × 2 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue

was purified by silica gel column chromatography (EtOAc−petroleum ether, 1:4 v/v) to give **37** (232 mg, 53%) as a white foam: R_f 0.75 (EtOAc−petroleum ether, 3:1 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.61 (br s, 1H, NH), 8.21 (d, 1H, J = 7.8 Hz, H-6), 8.12 (m, 2H, Ar), 7.58 (m, 1H, Ar), 7.44 (m, 2H, Ar), 6.09 (m, 1H, H-7′), 5.69−5.64 (m, 2H, H-5, H-1′), 4.80, 4.71 (AB, 2H, J = 12.9 Hz, CH_2OBz), 4.48 (d, 1H, J = 5.7 Hz, H-3′), 4.16, 3.89 (AB, 2H, J = 11.4 Hz, H-5′), 2.62 (m, 1H, H-8′), 2.42−2.35 (m, 2H, H-8′, H-2′), 0.90 (s, 9H, SiC(CH_3)$_3$), 0.81 (s, 9H, SiC(CH_3)$_3$), 0.08 (s, 3H, SiCH_3), 0.07 (s, 3H, SiCH_3), 0.05 (s, 3H, SiCH_3), 0.05 (s, 3H, SiCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.8 (*C*OAr), 166.0 (C-4), 150.3 (C-2), 140.6 (C-6), 133.6, 133.2, 130.3, 129.7, 128.5 (Ar, C-6′), 101.1 (C-5), 89.2 (C-1′), 83.3 (C-4′), 65.3, 65.1 (C-3′, *C*H$_2$OBz), 59.8 (C-5′), 44.4 (C-2′), 28.0 (C-8′), 26.0, 25.6 (SiC(*C*H$_3$)$_3$), 18.5, 17.9 (Si*C*(CH$_3$)$_3$), −4.5, −5.0, −5.2, −5.4 (SiCH$_3$); HRMALDI MS m/z (651.2898 [M + Na]$^+$, $C_{32}H_{48}N_2O_7Si_2$−Na$^+$ calcd 651.2892).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-2-Benzoyloxymethyl-8-hydroxy-1-hydroxymethyl-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]oct-2-ene (38). To a stirred solution of compound **37** (232 mg, 0.37 mmol) in anhydrous CH_3CN (2 mL) were added KF (0.215 g, 3.70 mmol) and 18-crown ether-6 (390 mg, 1.48 mmol), and the solution was stirred in a microwave reactor at 100 °C for 1 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (0−5%CH_3OH in CH_2Cl_2) to give **38** (61 mg, 41%) as a white foam: R_f 0.45 (MeOH−dichloromethane, 1:9 v/v); ^{1}H NMR (300 MHz, (DMSO-d_6) δ 11.29 (s, 1H, NH), 8.12 (d, 1H, J = 8.1 Hz, H-6), 7.95 (m, 2H, Ar), 7.67 (m, 1H, Ar), 7.53 (m, 2H, Ar), 6.03 (m, 1H, H-7′), 5.58 (d, 1H, J = 8.1 Hz, H-5), 5.48 (s, 1H, H-1′), 5.30 (d, 1H, J = 4.5 Hz, 3′−OH), 5.23 (t, 1H, J = 4.2 Hz, 5′−OH), 4.78, 4.71 (AB, 2H, J = 12.6 Hz, CH_2OBz), 4.36 (t, 1H, J = 4.5 Hz, H-3′), 3.94 (dd, 1H, J = 4.2, 12.3 Hz, H-5′), 3.76 (dd, 1H, J = 4.2, 12.3 Hz, H-5′), 2.57 (m, 1H, H-8′), 2.37 (m, 1H, H-2′), 2.18 (m, 1H, H-8′); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.1 (*C*OAr), 163.3 (C-4), 150.2 (C-2), 140.1 (C-6), 133.4, 133.2, 131.5, 129.6, 129.1, 128.8 (Ar, C-6′, C-7′), 100.4 (C-5), 88.3 (C-1′), 82.4 (C-4′), 64.7, 64.2, 57.7 (C-3′, *C*H$_2$OBz, C-5′), 43.2 (C-2′), 27.5 (C-8′); HRMALDI MS m/z (423.1172 [M + Na]$^+$, $C_{20}H_{20}N_2O_7$−Na$^+$ calcd 423.1163).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-2-Benzoyloxymethyl-1-(4,4′-dimethoxytrityloxymethyl)-8-hydroxy-6-(uracil-1-yl)-7-oxabicyclo-[3.2.1]oct-2-ene (39). DMT-Cl (0.155 g, 0.46 mmol) was added to a stirred solution of **38** (0.061 g, 0.15 mmol) in anhydrous pyridine (1 mL) and anhydrous CH_3CN (1 mL), and the mixture was stirred for 24 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (0−4% CH_3OH and 0.5% pyridine in CH_2Cl_2) to give **39** (82 mg, 77%) as a foam: R_f 0.50 (CH_3OH−dichloromethane, 7.5:92.5 v/v); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.10 (s, 1H, NH), 8.21 (d, 1H, J = 8.1 Hz, H-6), 7.79 (m, 2H, Ar), 7.57 (m, 1H, Ar), 7.42−7.16 (m, 11H, Ar), 6.84−6.76 (m, 4H, Ar), 6.13 (m, 1H, H-7′), 5.67 (s, 1H, H-1′), 5.41 (d, 1H, J = 8.1 Hz, H-5), 4.70−4.66 (m, 2H, CH_2OBz, H-3′), 4.56 (d, 1H, J = 12.6 Hz, CH_2OBz), 3.80−3.74 (m, 7H, OCH_3, H-5′), 3.68 (d, 1H, J = 10.8 Hz, H-5′), 2.70−2.42 (m, 3H, H-8′, H-2′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8 (*C*OAr), 163.6 (C-4), 158.8 (Ar), 150.2 (C-2), 144.3 (Ar), 140.4 (C-6), 135.1, 135.0, 133.5, 133.1, 130.2, 130.1, 129.7, 129.6, 128.5, 128.1, 127.3, 113.3 (Ar, C-6′, C-7′), 101.4 (C-5), 89.4 (C-1′), 87.5, 82.2 (C-4′, *C*Ar$_3$), 67.0, 65.0, 60.3 (*C*-3′, *C*H$_2$OBz, *C*-5′), 55.3 (OCH_3), 43.8 (C-2′), 27.4 (C-8′); HRMALDI MS m/z (725.2468 [M + Na]$^+$, $C_{41}H_{38}N_2O_9$−Na$^+$ calcd 725.2470).

Synthesis of (1*R*,5*R*,6*R*,8*S*)-2-Benzoyloxymethyl-8-(2-cyanoethoxy(diisopropylamino)phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-6-(uracil-1-yl)-7-oxabicyclo[3.2.1]oct-2-ene (40). To a stirred solution of compound **39** (0.122 g, 0.17 mmol) in anhydrous CH_2Cl_2 (1.5 mL) were added *N,N*-diisopropylethylamine (0.180 mL, 1.04 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (116 μL, 0.52 mmol). The mixture was stirred for 30 h and then CH_2Cl_2 (3 mL) added. The mixture was washed with a saturated aqueous solution of $NaHCO_3$ (2 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 3 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (0−2.5% CH_3OH and 0.5% pyridine in CH_2Cl_2) to give **40** (70 mg, 45%) as a white foam: R_f 0.80 (CH_3OH−dichloromethane, 7.5:92.5 v/v); ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 150.0, 149.5; ESI MS m/z (925.3543 [M + Na]$^+$, $C_{50}H_{55}N_4O_{10}P$−Na$^+$ calcd 925.3548).

Synthesis of (1*R*,7*S*,9*R*,10*R*,12*S*)-12-(*tert*-Butyldimethylilyloxy)-1-(*tert*-butyldimethylsilyloxymethyl)-5,6-bis(ethoxycarbonyl)-10-(uracil-1-yl)-11-oxatricyclo[7.2.1.0^{2,7}]dodeca-2.5-diene (41). A solution of the nucleoside **31** (160 mg, 0.31 mmol) in anhydrous toluene (2 mL) was added diethyl acetylendicarboxylate (0.490 mL, 3.07 mmol), and the solution was stirred in a microwave reactor at 150 °C for 2 h. The mixture was concenrated under reduced pressure, and the residue was purified by silica gel column chromatography (0−2.5% CH_3OH in CH_2Cl_2) to give **41** (160 mg, 75%) as a white foam: R_f 0.60 (MeOH−dichloromethane, 1:19 v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (s, 1H, NH), 8.11 (d, 1H, J = 8.0 Hz, H-6), 5.87 (d, 1H, J = 2.8 Hz, CH=C), 5.63 (d, 1H, J = 8.0 Hz, H-5), 5.50 (s, 1H, H-1′), 4.41 (d, 1H, J = 4.0 Hz, H-3′), 4.35−4.15 (m, 5H, 2 × CH_3CH_2, H-5′), 4.08 (m, 1H, H-7′), 3.68 (d, 1H, J = 11.6 Hz, H-5′), 3.20 (ddd, 1H, J = 4.4, 8.8, 23.6 Hz, =C−CH_{2a}-C=), 2.92 (ddd, 1H, J = 2.4, 11.2, 23.6 Hz, =C−CH_{2b}-C=), 2.42−2.34 (m, 2H, H-8′$_b$, H-2′), 1.79 (m, 1H, H-8′$_a$), 1.38−1.20 (m, 6H, 2 × CH_3CH_2), 0.95 (s, 9H, SiC(CH_3)$_3$), 0.88 (s, 9H, SiC(CH_3)$_3$), 0.22 (s, 3H, SiCH_3), 0.19 (s, 3H, SiCH_3) 0.10 (s, 6H, SiCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.2, 166.6 (CO_2Et), 163.3 (C-4), 150.1 (C-2), 140.1, 139.8 (C-6, C-6′), 135.6, 126.4 (O_2CC=*C*CO_2), 121.4 (*C*H=C), 101.0 (C-5), 90.6 (C-1′), 83.8 (C-4′), 68.2 (C-3′), 61.3 (CH_3CH_2), 60.7 (C-5′), 43.2 (C-2′), 30.5, 30.0 (C-7′, C-8′), 27.6 (=C-*C*H$_2$−C=), 26.2, 25.7 (SiC(*C*H$_3$)$_3$), 18.6, 17.9 (Si*C*-(CH$_3$)$_3$), 14.2 (*C*H$_3$CH$_2$) −4.8, −4.9, −5.1, −5.3 (SiCH$_3$); HRMALDI MS m/z (713.3250 [M + Na]$^+$, $C_{34}H_{54}N_2O_9Si_2$−Na$^+$ calcd 713.3260).

Synthesis of Oligodeoxynucleotides. Oligonucleotide synthesis was carried out on an automated DNA synthesizer following the phosphoramidite approach. Synthesis of oligonucleotides **42**−**50** was performed on a 0.2 μmol scale by using the amidites **29**, **33**, **35**, and **40** 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 **29**, **33**, **35**, and **40**, a prolonged coupling time of 30 min was used, and for **29** and **40**, pyridinium hydrochloride was used as the activator instead of 1*H*-tetrazole in all other cases. Coupling yields for all 2-cyanoethyl phosphoramidites were >98%. The 5′-*O*-DMT-protected oligonucleotides were removed from the universal solid support by treatment with concentrated ammonia at 55 °C for 20 h. The oligonucleotides were purified by reversed-phase HPLC on a Waters 600 system using a X_{terra} prep MS C_{18}; 10 μm; 7.8 × 150 mm column; gradient of buffer (0.05 M triethylammonium acetate) in 75% CH_3CN(aq); 0−70% buffer, 38 min; 70−100% buffer, 7 min; 100% buffer, 10 min. All fractions containing 5′-*O*-DMT-protected oligonucleotide (retention time 20−30 min) were collected and concentrated. The products were detritylated by treatment with an 80% aqueous solution of acetic acid for 20 min, and finally isolated by precipitation with ethanol at −18 °C overnight. MALDI-MS [M − H]$^-$ gave the following results (found/calcd): **43** (2810.2/2811.9); **44** (2925.9/2928.0); **45** (2805.6/2807.9); **46** (2919.0/2916.0); **47** (2804.0/2803.9); **48** (2907.2/2904.0); **49** (2811.8/2807.9); **50** (2918.9/2916.0); **53** (4755.0/4758.2); **54** (4873.3/4874.2).

Melting Experiments. UV melting experiments were carried out on a UV spectrometer. For the duplex experiments, the

samples were dissolved in a medium salt buffer containing Na_2HPO_4 (5 mM), NaH_2PO_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 versus temperature curve. All melting curves were found to be reversible. All determinations are averages of duplicates. For the triplex experiments, a buffer containing 10 mM sodium cacodylate, 150 mM NaCl, 10 mM $MgCl_2$, pH 6.0 using 1.0 μM concentration of the target duplex and 1.5 μM concentrations of the TFO sequences.

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CD Spectroscopy. CD spectra were recorded in the same medium salt buffer as in the UV melting experiments with 3.0 μM concentrations of the two complementary sequences.

Quantum Mechanical Calculation. Ab initio calculations were carried out using the Gaussian 03 program.[37] Boat and chair conformations of the six-membered ring of the unprotected form of **25** were generated in a two-step procedure. First, a constrained geometry optimization was performed with constraints ensuring idealized chair and boat conformations. The 5-hydroxy group was constrained in a trans geometry, and the 3′-hydroxy group was rotated so as not to clash with the six-membered ring. Second, the two geometries obtained in the first step were subjected to free geometry optimizations. Geometry optimizations were carried out using Hartree−Fock theory (HF) with the 6-31G* basis set. Single-point energies of optimized conformations were determined using second-order Møller−Plesset theory (MP2) with the cc-pVTZ basis set.

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Supporting Information Available: General introduction to the Experimental Section. Selected NMR spectra. Melting curves for the triplex study. This material is available free of charge via the Internet at http://pubs.acs.org.