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An α-D-Configured Bicyclic Nucleoside Restricted in an *E*-type Conformation: Synthesis and Parallel RNA Recognition

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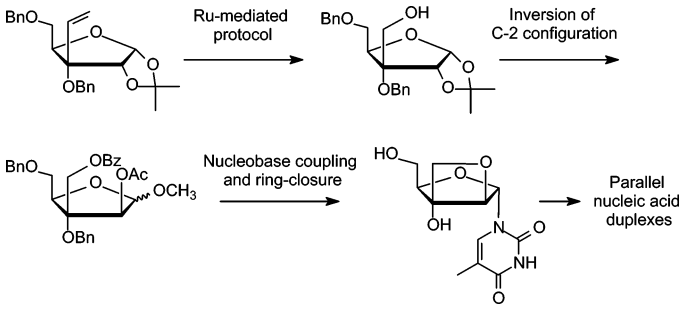

An α-D-*arabino* configured bicyclic nucleoside strongly restricted in an *E*-type conformation by a 2′-3′-fused oxetane ring is synthesized. Several synthetic strategies toward the target compound are described, and the successful preparation from a D-xylose derivative is based on a ruthenium-mediated cleavage of a double bond, an S_N2-inversion at the 2-position to give an *arabino*-configuration, nucleobase coupling, and finally ring closure to give the oxetane ring. The *E*-type conformation is confirmed by molecular modeling and NMR. The nucleoside is incorporated into short α-DNA sequences. In a mixed pyrimidine context, these recognize complementary parallel RNA-sequences with mainly increased affinity and complementary parallel DNA-sequences with decreased affinity. The present bicyclic analogue represents the first conformationally restricted α-DNA-analogue to improve nucleic acid recognition in mixmers with α-DNA monomers.

Introduction

Conformational restriction of synthetic nucleic acids by the use of nucleoside analogues with bi- or tricyclic carbohydrate moieties has been an excellent tool in nucleic acid chemical biology.[1,2] The induced control of geometry has led to stabilized nucleic acid duplexes and other structural motifs with great potential in the development of therapeutics[1−3] and diagnostics,[3] and for the design of functional nucleic acid architectures.[4] The most intriguing example is LNA (locked nucleic acid) in which the nucleoside building blocks (as the thymine monomer **1**, Figure 1) are locked in *N*-type conformations,[3,5] hereby dictating an overall A-type duplex formation.[6,7] Thus, the incorporation of single LNA monomers into an oligodeoxynucleotide induces a conformational shift in the neighboring 2′-deoxynucleotides toward the *N*-type conformation leading to strongly stabilized duplexes with antiparallel DNA and RNA complements.[5,6] α-Configured 2′-deoxy nucleic acid sequences (α-DNA) are well-known for their recognition of complementary DNA and RNA sequences forming stable parallel stranded duplexes.[8] This rather unusual structural motif can be

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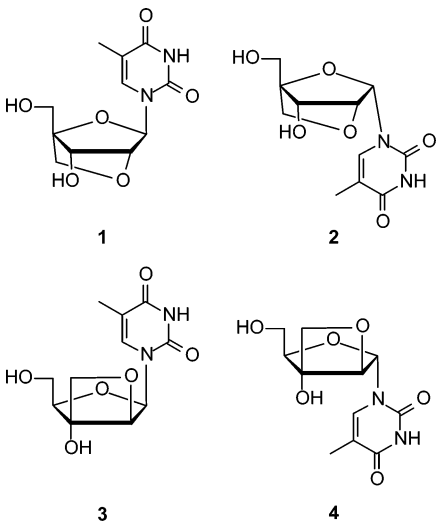

FIGURE 1. α- and β-configured bicyclic thymidine analogues.

further stabilized by replacing phosphordiester moieties in α-DNA with cationic phosphoramidate analogues.[9] Conformational restriction of the sugar moieties of α-DNA has not led to the same conclusions as with ordinary DNA. We have introduced the α-D-configured analogue of LNA (α-LNA, see thymine monomer **2**, Figure 1) as an α-DNA analogue with locked *N*-type nucleoside conformations.[10] Sequences of pyrimidine monomers were synthesized and studied,[10] whereas mixed sequences including purines have been evaluated recently in a mirror-image study.[11] Fully modified α-LNA sequences displayed an unprecedented recognition of parallel complementary RNA sequences whereas complementary DNA-sequences were not recognized.[10,11] On the other hand, mixmers of α-LNA and α-DNA displayed a low affinity for RNA.[10] This observation can most probably be attributed to the inability of α-DNA monomers to adopt *N*-type conformations, thus creating incoherency between *N*- and *S*-type conformations within the same sequence.[10] This led to the conclusion that a locked *N*-type conformation is not the solution for making a stabilized parallel duplex formation with the option of fine-tuning the thermal stability in mixmer sequences.

The α-L-configured analogue of LNA (α-L-LNA) has been demonstrated to be an excellent mimic of DNA.[12] Thus, α-L-LNA mixmers formed high-affinity antiparallel duplexes with complementary DNA displaying overall B-type conformation and with complementary RNA displaying an intermediate conformation.[12,13] Therefore, we recently envisioned and proved that the anomer of α-L-LNA, i.e., β-L-LNA is a good overall mimic of α-DNA.[11] In a complete mirror-image study, β-L-LNA sequences were proved to recognize both complementary RNA and DNA sequences in a parallel stranded mode and with affinities comparable to what was found for the corresponding parallel α-LNA:RNA duplex.[11] However, synthesis of β-L-LNA monomers has not been realized and mixmers of β-L-LNA and α-DNA have not been studied. Recently, Keller and Leumann introduced a true *S*-type α-DNA mimic with a perfect γ-torsion[7] due to a tricyclic carbohydrate skeleton.[14] However, in mixmers with α-DNA this was found to give slightly decreased affinity toward complementary DNA and RNA.[14]

As a third alternative to nucleoside analogues with strong restrictions in *N*- or *S*-type conformations, bicyclic nucleosides restricted toward the intermediate *E*-type (or O4′-*endo*) conformations[7] have been introduced.[15−17] As the prime example, the nucleoside **3** with an 2′-*O* to 3′-*C* oxetane ring (Figure 1) has been shown to increase the affinity toward complementary RNA and DNA sequences in both fully modified sequences and in mixmers with DNA.[16,18] Recently, we developed an improved synthesis of **3** in order to undertake further biological studies.[19] Also the 3′-azido-3′-deoxy analogue of **3** as well as its α-configured epimer have been synthesized and investigated as potential antiviral compounds.[20] Herein, we introduce the corresponding epimer of **3**, i.e., the [3.2.0]-bicyclic α-D-configured nucleoside **4** as the first *E*-type mimic of α-DNA. Here, we expected a nucleoside analogue that, in contrary to the *N*-type mimic **2**, can work in mixmers with α-DNA to increase the scope of parallel nucleic acid recognition. For immediate comparison with other studies and for synthetic convenience, we decided to concentrate on exclusively the thymidine derivative **4** for this study and postpone the preparation of other pyrimidine or purine analogues to subsequent studies.

Results

Retrosynthetic Considerations. Several possible routes toward the α-configured [3.2.0]bicyclic nucleoside **4** were evaluated, as indicated in Figure 2 on the level of an abridged retrosynthetic analysis. In principle, the

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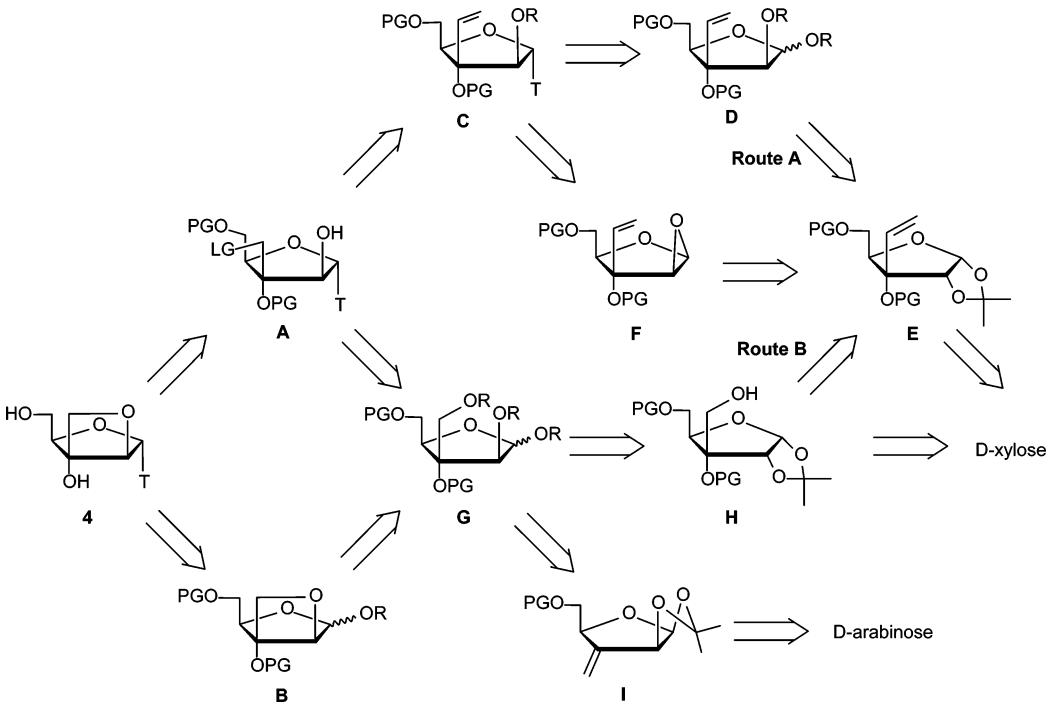

FIGURE 2. Retrosynthetic analysis of **4**. R = varying protons or protecting groups. PG = stable protecting groups for 5′- and 3′-alcohols. LG = leaving group. T = thymin-1-yl.

target compound might be accomplished by two fundamentally different final steps, either with the oxetane ring formation by a nucleophilic ring-closure as the last step on the appropriately prepared α-nucleoside **A**, or alternatively, nucleobase coupling on the appropriately prepared bicyclic furanose derivative **B**. The latter route was immediately refused as attempts on Vorbrüggen type coupling reactions[21] with conformationally restricted bicyclic carbohydrate substrates on several occasions have been shown to give ring-opening reactions and release of ring strain rather than glycosidic coupling reactions.[22] Furthermore, mixtures of α- and β-configured products would be expected in this case. Therefore, we concentrated on the possible routes toward the key intermediates of type **A**, and the initial route to be considered (route A, Figure 2) was modeled after the previously published synthesis of the β-configured analogue **3**.[16] Thus, the leaving group in **A** precedes a 3′-*C*-hydroxymethyl group that might be obtained by oxidative cleavage of a vinyl group in **C**. This compound should be conveniently accessible by nucleobase coupling from **D**. However, the compound **D** should have *arabino*-configuration in order to take advantage of anchimeric assistance from a suitable 2-*O*-protecting group. Opening of the isopropylidene ring followed by inversion of configuration at C-2 of **E** was anticipated to give the required **D**. **E** is well-known to be readily obtainable by the stereoselective Grignard addition of a vinyl group to an appropriately protected 3-ulofuranose of D-xylose.[16] Alternatively, **C** could be obtained from **E** via the 1,2-anhydro-β-D-arabinofuranose derivative **F** based on similar transformations reported by Chow and Danishefsky.[23] Thus, the salient feature of route A is the use of the vinyl group as a precursor for the hydroxymethyl group as well as stable protecting groups of the 3′-*O* and 5′-*O* positions until the synthesis of nucleoside **A**.

Route B differs strategically from route A in the sense that the oxidative cleavage of the double bond precedes the nucleobase coupling. Thus, compound **A** should be available by stereoselective base coupling from **G**. However, **G** must have *arabino*-configuration and should be accessible with a protected hydroxymethyl group at C-3 in correct stereochemistry from **H** by inversion of configuration at C-2. The hydroxymethyl group of **H** could again be formed via oxidative cleavage of the double bond in the precursor **E**. As a last alternative, a route through the 3-methylene furanose **I** was considered. This would be accessible in fewer steps starting from D-arabinose,[24] and hereby the route would resemble the successful route toward the 3′-azido-3′-deoxy analogues of **3** and **4**.[20] However, the route through **I** was not pursued as it is known that dihydroxylation of olefin **I** (PG = TBDPS) proceeds exclusively from the β-face of the ring[24] rather than (more expectedly) from the α-face that is required in our case. Finally, it might be considered if **H** could be obtained directly from D-xylose, i.e., through the 3-ulofuranose thereof, with a nucleophilic attack of another hydroxymethyl precursor rather than the vinyl group. Nevertheless, any potential $ROCH_2M$ organometallic reagent or anything like it would lead to an alternative set of protecting groups to be handled. The vinyl group is an obvious and inert precursor for the hydroxymethyl

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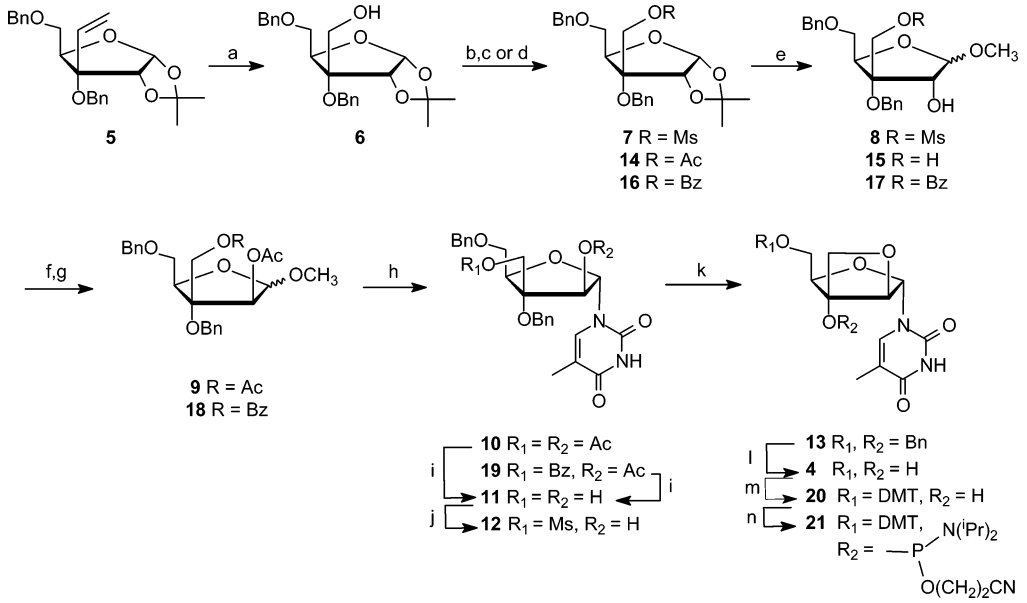
SCHEME 1. Synthesis of Nucleoside 4[a]



[a] Reagents and conditions: (a) $RuCl_3 \cdot xH_2O$, $NaIO_4$, H_2O, EtOAc, CH_3CN, then $NaBH_4$, H_2O, THF, then $NaIO_4$, then $NaBH_4$, ref 19 (72%); (b) MsCl, pyridine (94% **7**); (c) Ac_2O, pyridine (98%, **14**); (d) BzCl, pyridine (**16**); (e) CH_3COCl in $CH_2Cl_2/CH_3OH/H_2O$ (66% **8**; 81% **15**); (f) Tf_2O, pyridine, CH_2Cl_2, −30 to 0 °C; (g) CsOAc, 18-crown-6, DMF, 95 °C, (46% **18** over four steps from **6**); (h) thymine, *N,O*-bis(trimethylsilyl)acetamide, CH_3CN, TMS-triflate (68% **19**); (i) $NaOCH_3$, MeOH (90% **11** from **19**); (j) MsCl, pyridine, −40 °C to rt (84% from pure **11**; 10% over five steps from **8**); (k) NaH, dioxane, 50 °C (98%); (l) H_2, $Pd(OH)_2/C$ (98%); (m) 4,4′-dimethoxytrityl chloride, pyridine; (n) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(iPr)_2$, CH_2Cl_2 (79% over two steps).

group, and any other opportunities were postponed until full coverage of the convenient starting material **E**.

Initial Attempts. Our first approach for the synthesis of **4** was highly motivated by the original synthesis of the β-analogue **3**[16] and, therefore, followed the retrosynthetic route A. Hence, the dibenzylated 3′-*C*-vinyl compound **5** (corresponding to **E** (PG = Bn), Figure 2) was obtained from a D-xylose derivative via a stereoselective Grignard reaction in a well-known and high yielding five-step procedure.[16] Three different strategies for the inversion of C-2 configuration were tried, namely (i) an S_N2 reaction, (ii) 1,2-anhydro formation (through **F**), and (iii) an oxidation−reduction procedure. The latter strategy gave the best results, and a material corresponding to **D** was obtained. However, after successful coupling to give **C**, the oxidative cleavage to give **A** was troublesome, and in the end, **4** could be obtained in a maximum of only 2.7% overall yield from **5**. All aspects of our efforts toward **4** through Route A are covered in the electronic Supporting Information.

Synthesis of 4 by Route B. After the efforts following route A, it became clear that the major bottleneck here (even more problematic than it was in the original synthesis of the β-analogue **3**[16]), was the oxidative cleavage of the vinyl group to a hydroxymethyl group using a two-step $OsO_4-NaIO_4/NaBH_4$ protocol.[25] Hence, we decided to perform the problematic oxidative cleavage step in the beginning of the reaction sequence with the conveniently obtained compound **5**[16] as the substrate (Scheme 1). Our attempts toward oxidative cleavage of the double bond of **5** using the standard osmium tetraoxide mediated protocols ($OsO_4-NaIO_4/NaBH_4$ or OsO_4−NMO followed by $NaIO_4/NaBH_4$)[25,26] as well as ozonolysis-reduction protocols[27] were unsuccessful.[28] Nevertheless, we were successful in developing a new general and efficient ruthenium-catalyzed protocol for the oxidative cleavage of monosubstituted alkenes to yield exclusively (one carbon shorter) primary alcohols. Thus, using ruthenium tetraoxide (made in situ from $RuCl_3 \cdot xH_2O$ and $NaIO_4$ as a co-oxidant) for the oxidation, followed by an additional sodium borohydride reduction step before the diol-cleavage and reduction successfully afforded the conversion of **5** to the hydroxymethyl product **6** in 72% yield (Scheme 1). This reaction also paved the way for a highly improved synthesis of the known β-configured bicyclic nucleoside **3**, and we have recently published the general ruthenium protocol and the significantly improved synthesis of **3** in a note.[19]

Having achieved the desired goal of efficient conversion of **5** to **6**, we attempted the synthesis of the target nucleoside **4** as depicted in Scheme 1. We decided to mesylate the hydroxy group of **6** considering our need for a leaving group for the creation of an oxetane ring as shown in Figure 2. Based on previous experiences, we hoped that the mesylate group would be stable in the conditions needed for inversion of C-2 configuration and for the coupling of the nucleobase. Thus, in the preparation of α-L-LNA monomers, mesylates on a 5-*O* position

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and a 4-*C*-hydroxymethyl position have been found to be stable under the conditions needed for an S_N2 inversion of C-2 configuration by the use of a 2-*O*-trifluoromethanesulfonate.[12,29] Accordingly **6** was mesylated in pyridine to give monomesylate **7** in a high yield. Treatment of **7** with methanolic hydrochloric acid afforded the anomeric mixture (β/α = 2.3:1) of methyl furanosides **8** in 66% yield. The inversion of configuration at C-2 was then attempted by conversion of **8** into the corresponding 2-*O*-trifluoromethanesulfonate and subsequent reaction with cesium acetate in DMF in the presence of 18-crown-6. This, however, resulted in a complex mixture of compounds which was thought to be a mixture of *arabino*-configured diacetate **9** and an unidentified compound. Thus, under these reaction conditions, the mesylate group participated in an S_N2 reaction with cesium acetate. Efforts to further separate the mixture by column chromatography were not successful and coupling of this mixture with thymine using the Vorbrüggen method[21] was performed to give an inseparable mixture of α-nucleoside **10** and an unidentified nucleoside. Deacetylation of the diacetate **10** to give **11** along with an unidentified product, followed by selective mesylation of the primary hydroxyl group gave a complex mixture. Fortunately, we were able to separate this complex mixture by column chromatography and obtained pure required monomesylate **12** in 10% overall yield from **8**. Ring closure by the use of sodium hydride gave **13** and debenzylation afforded the target bicyclic nucleoside **4** in 96% yield over the two steps. Thus, we were successful in completing the synthesis of target nucleoside **4** but the reaction sequence was marred by the formation of unexpected products presumably due to the use of mesyl group to protect (and later activate) the primary hydroxyl group resulting in a very low overall yield of 2.9% of **4** from **5**. As the mesyl group got replaced by an acetyl group during the inversion step, we decided to protect the primary hydroxyl group of **6** by an acetyl group. Thus, **6** was converted to monoacetate **14** in 98% yield and subsequent treatment of **14** with methanolic hydrochloric acid resulted in required methanolysis to yield the methyl ribofuranoside. However, the acetyl group could not survive the reaction conditions and we got deacetylated methyl ribofuranosides **15** along with unidentified side products presumably due to alternative furanose formation. Hence, it became clear that neither the acetyl was the right protecting group for further elaborations and this route was therefore not pursued further.

Careful perusal of the reaction sequence revealed that a benzoyl group should be able to survive the acidic methanolysis as well as the inversion conditions. Accordingly, benzoylation of hydroxyl group of **6** to give **16** followed by acidic methanolysis afforded the anomeric mixture of methyl furanosides **17**. NMR of the crude **17** revealed the anomeric mixture to be approximately in a β/α ratio of 2.7:1. Inversion of C-2 configuration was again attempted in a two step procedure converting the 2-hydroxyl group to a strong leaving group followed by an S_N2 reaction by cesium acetate taking advantage of the "cesium effect".[30] Hereby, we got the desired arabinofuranoside **18** in 46% yield over four steps from **6**. Taking into consideration that α-trisubstituted secondary alcohols (a neopentyl-type system) are among the poorest candidates for an S_N2 inversion process due to the steric hindrance and the competing S_N1 reaction, this was a very satisfying result. In our first strategy (route A, Figure 2), low yields were obtained with the 3-vinyl analogue of **17** (β/α = 2.5:1) as the substrate for this process, as only the α-anomer participated in the inversion reaction (see the Supporting Information). Hence, the yield at the inversion step improved considerably by replacing the vinyl group with the benzoyloxymethyl group, and moreover, **18** was found to be an anomeric mixture approximately in an $\alpha:\beta$ ratio of 1.1:1 indicating clearly that both the anomers of **17** responded to the inversion conditions. Coupling of **18** with thymine using the Vorbrüggen method[21] yielded the α-D-*arabino*-configured nucleoside **19** in 68% yield. Deacetylation and debenzoylation was performed in a single step using sodium methoxide to give the diol **11** in 90% yield. The selective mesylation of **11** to give **12**, oxetane ring formation to give **13** and debenzylation afforded the target bicyclic nucleoside **4** in 81% yield over the three steps. The overall yield of **4** from **5** by this final strategy was 16.3% as compared to overall yields of 1.3−2.7% (route A, see the Supporting Information) and 2.9% (by using a mesyl group instead of a benzoyl group in route B).

Conformation of Nucleoside 4. Nucleoside conformations are conveniently defined by the pseudoration angle.[7,31] Thus, the *N*-type conformation corresponds to a pseudoration angle $P \approx 18°$, the *S*-type to $P \approx 162°$, whereas *E*- and *W*-type (O4′-*endo* and O4′-*exo*) conformations correspond to $P \approx 90°$ and 270°, respectively.[7,31] A pseudorotation energy profile for **4** was calculated by ab initio calculations at the MP2/cc-pVTZ level (see Figure 3). Two local energy minima were found in the pseudorotation profile for this nucleoside ($P \approx 110°$ and 270°) corresponding to *E*- and *W*-type conformations, respectively, and the minimum at $P \approx 110°$ is 6.0 kcal/mol lower in energy than the $P \approx 270°$ minimum. Using a generalized Karplus equation,[32−34] we found $J_{H1'-H2'}$ = 0.6 and 3.9 Hz for the two low energy sugar puckers, respectively. Experimentally, H-1′ displays a singlet in the ^{1}H NMR spectrum of **4** corresponding to a $J_{H1'-H2'} < 1.0$ Hz, and this shows that **4** exclusively adopts an *E*-type furanose conformation in solution without repuckering to the *W*-conformation. The pseudorotation profile is characterized by high energy barriers rendering pseudorotation impossible. The conformation with a planar furanose is 4.9 kcal/mol higher in energy than the P ≈ 110° geometry. An unconstrained geometry optimization (MP2/6-31G*) gave a structure with $P = 97°$ and a puckering amplitude $\Phi_{max} = 33°$ (see Figure 3, structure inserted). Therefore, it is confirmed that the bicyclic nucleoside **4**

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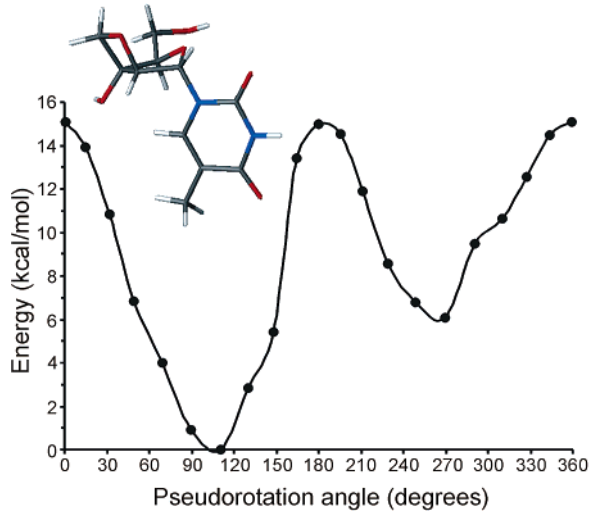

FIGURE 3. MP2/cc-pVTZ potential energies as a function of pseudorotation angle P for **4**. All energies are given relative to the $P = 110°$ conformation. Inset: the geometry-optimized (MP2/6-31G*) structure displaying an *E*-type conformation with $P = 97°$.

like its β-anomer **3**[16] is strongly conformationally restricted in an *E*-type (O4′-*endo*) conformation and only samples conformations near this global energy minimum.

Preparation of Oligonucleotides and Thermal Denaturation Studies. To incorporate the [3.2.0]bicyclic nucleoside monomer into oligonucleotides, **4** was converted to the appropriately protected phosphoramidite derivative (Scheme 1). Thus, the primary alcohol function of **4** was easily protected with the DMT (4,4′-dimethoxytrityl) group to afford **20**, which was eventually transformed into the corresponding 3′-*O*-phosphoramidite building block **21**. For the automated solid-phase synthesis of oligonucleotides by the phosphoramidite approach,[35] the bicyclic α-nucleoside monomeric building block **21** was used in combination with the corresponding unmodified α-2′-deoxynucleoside thymine and 5-methylcytosine phosphoramidites, which were obtained according to literature methods.[36,37] Hence, modified and unmodified α-DNA sequences (**22**−**30**, Tables 1 and 2) were obtained with >98% stepwise coupling yields by using tetrazole activation and coupling times of 10−15 min for **21**. All sequences were obtained on universal CPG support (Biogenex/Glen Research) using the DMT-*ON* mode. This allowed synthesis of fully α-configurated sequences after cleavage from the solid support using LiCl in aqueous ammonia. The oligomers **22**−**30** were purified by using reversed-phase HPLC yielding products with >90% purity. The compositions of all sequences were verified from their MALDI mass spectra.

For the synthetic convenience and the direct comparison with the results obtained for α-LNA (monomer **2**), only 10-mer and 14-mer oligothymidylate sequences (Table 1) and a mixed 10-mer pyrimidine sequence (Table 2) were employed for this study. The hybridization between the modified α-DNA sequences and unmodified

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TABLE 1. Hybridization Data of Modified and Unmodified Oligothymidylate α-DNA Sequences

	ODN sequences[a]	DNA (dA_{14}) T_m (ΔT_m)/°C[b]		RNA (rA_{14}) T_m (ΔT_m)/°C[b]	
22	5′-αT_{14}	32.0		43.0	
23	5′-αT_7**X**T_6	30.5 (−1.5)[c]	(−6.5)[e]	46.0 (+3.0)[c]	(−8.0)[e]
24	5′-αT_5**X**$_4T_5$	24.0 (−2.0)[c]	(−1.5)[e]	33.0 (−2.5)[c]	(−4.6)[e]
25	5′-αT_{10}	18.0		33.5	
26	5′-α**X**$_{10}$	<10		22.0 (−2.1)[d]	(+1.2)[e]

[a] α-DNA sequences with **X** = **4**. [b] Melting temperatures obtained from the maxima of the first derivatives of the melting curve (A_{260} vs temperature) recorded in a buffer containing 10 mM Na_2HPO_4, 100 mM NaCl, 0.1 mM EDTA, pH 7.0 using 1.0 μM concentrations of each strand. Values in parentheses show the changes in T_m values per modification compared with the reference strands. [c] Compared with **22**. [d] Compared with **25**. [e] These values refer to the corresponding changes in T_m values per modification obtained with α-LNA, i.e., **X** = **2**.[10]

DNA and RNA sequences were studied by thermal denaturation examination (Tables 1 and 2). The oligothymidylate sequences **22**−**26** were mixed with complementary dA_{14} and rA_{14} and melting temperatures of the complexes were determined. The affinity of a 14-mer α-DNA sequence **22** toward DNA was slightly decreased by the incorporation of the bicyclic monomer as in **23** and **24**, for which a drop in melting temperature of 1.5−2.0 °C for each modification was obtained. A fully modified decamer α-DNA sequence **26** cannot recognize complementary DNA. With complementary RNA the situation is different. Thus, with one bicyclic monomer incorporated in an otherwise unmodified α-DNA sequence, **23**, the affinity increases significantly with 3 °C. However, a fully modified sequence, **26**, displays a decreased affinity toward RNA, and the sequence with four bicyclic entities in the middle of a 14-mer α-DNA sequence, **24**, displays a similar decrease in affinity.

A mixed pyrimidine sequence allows a verification of a parallel recognition mode. Hence, the unmodified α-DNA sequence **27** displays the earlier established[8,10] high affinity for complementary parallel DNA and RNA. One bicyclic moiety, **28**, leads to a small decrease in the affinity for the parallel DNA sequence whereas three or six bicyclic moieties, **29** or **30**, result in a more pronounced destabilization. On the other hand, when the same sequences were mixed with complementary RNA, a stabilization of the parallel duplex is seen with one or six bicyclic moieties, **28** or **30**, but peculiarly, a small destabilization with three bicyclic moieties, **29**. All mixed pyrimidine sequences **27**−**30** were also mixed with complementary antiparallel DNA and RNA sequences, and as expected,[8,10] no melting temperatures above the detection limit of 10 °C were observed.

Tables 1 and 2 display also the corresponding changes in melting temperature obtained with α-LNA monomers (i.e., **2**) as the synthetic monomer in α-DNA instead of the [3.2.0]bicyclic monomer **4** in similar sequences. As evident from a direct comparison, the [3.2.0]bicyclic moiety leads to a less pronounced destabilization of the parallel α-DNA:DNA duplexes than does the α-LNA monomer. The effect of the [3.2.0]bicyclic moieties on α-DNA/RNA duplexes is even more favorable. Thus, the bicyclic monomer **4** increases the RNA-affinity though, apparently, dependent on sequence composition. In other words, the cooperativity between **4** and the α-DNA

TABLE 2. Hybridization Data of Modified and Unmodified Mixed Pyrimidine α-DNA Sequences

		DNA (p) dGAGGAAGAAA		RNA (p) rGAGGAAGAAA	
	ODN sequences[a]	T_m (ΔT_m)/°C[b]		T_m (ΔT_m)/°C[b]	
27	5′-α-^{m}CT^{m}C^{m}CTT^{m}CTTT	43.0		32.0	
28	5′-α-^{m}CT^{m}C^{m}CT**X**^{m}CTTT	42.0 (−1.0)	(−12.5)[c]	35.0 (+3.0)	(−6.0)[c]
29	5′-α-^{m}C**X**^{m}C^{m}C**X**T^{m}C**X**TT	34.0 (−3.0)		30.0 (−0.7)	
30	5′-α-^{m}C**X**^{m}C^{m}C**XX**^{m}C**XXX**	19.0 (−4.0)	(<−5.5)[c]	37.0 (+0.8)	(−1.7)[c]

[a] α-DNA sequences with **X** = **4**. [b] See Table 1. Values in parentheses show the changes in T_m values per modification compared with the reference strand **27**. [c] These values refer to the corresponding changes in T_m values per modification obtained with α-LNA, i.e., **X** = **2**.[10]

monomers is functional and mixmers with high RNA-affinity can be obtained, whereas mixmers between α-LNA and α-DNA monomers let to largely destabilized duplexes.[10] On the other hand, the fully modified sequence of 10 [3.2.0]bicyclic monomers **26** has a low RNA-affinity, whereas the corresponding α-LNA sequence displayed an RNA-affinity much higher than the corresponding α-DNA,[10] as evident also in mixed sequences.[10,11]

Discussion

Several different approaches were examined toward the synthesis of the target [3.2.0]bicyclic α-nucleoside **4**. In the end, the route B (see Figure 2 and Scheme 1) starting from **E** (i.e., **5**, originally obtained from D-xylose)[16] through intermediates **H**, **G** and **A** afforded **4** in a high 16% overall yield. All investigated strategies applied the vinyl group as a strategically "protected" hydroxymethyl group. In the end, the oxidative cleavage of the vinyl group was the major bottleneck for the successful preparation of **4**, but this key point was successfully passed by our development of a ruthenium-based four step reaction sequence.[19] It is our expectation that this sequence being generally applicable for the conversion of terminal alkenes to one carbon shorter primary alcohols can be a general tool and a good alternative to existing methods in other aspects of synthetic organic chemistry. The limiting step of our synthesis of **4** was hereafter the inversion of C-2-configuration relying on a two-step procedure giving the *arabino*-furanoside **18** from the corresponding *ribo*-furanoside **17**. On the other hand, this is by far the most convenient of the inversion reactions investigated in the present study. Thus, the *arabino*-furanoside **18** was formed as the only product and the handling of stereoisomeric mixtures of nucleosides (as in route A; see the Supporting Information) was thereafter avoided. Furthermore, this synthetic strategy is not based on the pyrimidine-mediated conversion of C-2′ configuration following the so-called anhydro approach[38] that has been used in many occasions for the preparation of *arabino*-configured nucleoside derivatives with pyrimidines as the nucleobase[39] including several bicyclic nucleosides.[12,15,16,19] That strategy leaves behind the preparation of the purine analogues needing tedious conversions of C-2′-configuration as the one present herein. In our strategy, therefore, the purine analogues could be expected to be formed by more or less equal efficiency, and compound **18** is therefore a key compound for the preparation of all analogues of **4** with different nucleobases.

The present hybridization data of modified α-DNA sequences can lead to some conclusions about the status of nucleic acid recognition by parallel duplex formation. Thus, with **4** as the first *E*-type conformational α-DNA mimic, some immediate comparisons between different conformations in α-DNA can be performed. From our former studies with α-LNA it is evident that the conformational behavior of the nucleosides in α-DNA and in DNA is very different.[10,11] For standard *β*-configured oligonucleotides and antiparallel duplexes, the strong *N*-type mimic LNA always leads to duplex stabilization also in mixmers.[3,5,6] In other words, the LNA monomers can force neighboring 2′-deoxynucleotides into *N*-type conformations and the overall duplex toward an A-type duplex.[5,6] This was absolutely not possible in parallel duplexes of α-DNA/DNA or α-DNA/RNA. In mixmers with α-DNA, the strong *N*-type mimic α-LNA always leads to significant duplex destabilization.[10] On the other hand, strong parallel recognition was found with a complete α-LNA sequence, however, in the formation of a very different extended duplex structure.[40] From the present study, it can be concluded that the *E*-type conformation is compatible with α-DNA nucleosides in mixmers. Thus, the incorporation of **4** into otherwise unmodified α-DNA sequences leads to stabilization of the α-DNA/RNA duplex in most cases. On the other hand, the corresponding α-DNA/DNA parallel duplex is destabilized with the *E*-type mimic, and fully modified duplexes (sequence **26** and complements) are in general not stable. Also, this result is opposed to the corresponding results with the *β*-anomeric *E*-type mimic **3** incorporated in standard DNA/DNA or DNA/RNA duplexes, where stabilizations were generally observed with both mixmers of **3** and 2′-deoxynucleotides and with fully modified sequences.[16,18] Finally, conformationally restricted or even locked *S*-type mimics have not given the same stabilization of the standard *β*-configured and antiparallel duplexes as it might have been expected. However, all bi- or tricyclic *β*-nucleoside *S*-type mimics studied[41,42] suffers from other problems, including sterical problems with standard (B-type) duplex geometry.[42,43] The same

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might be evident for the few α-configured *S*-type mimics studied so far, i.e., the α-D-*arabino*-2′-*O*-methylthymidine,[44] a [4.3.0]bicyclic nucleoside[45] as well as the bicyclic[46] and tricyclic nucleoside analogues[14] from Leumann and co-workers. In the latter example, however, this was met by a strong restriction of not only the sugar conformation but also of the torsion angle γ.[14] Nevertheless, and despite the obvious need for experimental evidence, one might argue that the gain of a strong conformational restriction of α-DNA into an *S*-type conformation could be relatively small, as the unmodified α-DNA monomers are already strongly restricted toward this conformation by the combined contributions from anomeric and gauche stereoelectronic effects.[47,48]

Finally, the parallel nucleic acid recognition found for β-L-LNA is interesting. Though not displaying *S*-type sugar conformations, this LNA-stereoisomer is certainly an α-DNA and not an α-RNA mimic.[11] Therefore, mixmers of β-L-LNA and α-DNA, perhaps even combined with our *E*-type mimic **4**, might be favorable for parallel duplex formation and fine-tuning of duplex stability. The combination of several modifications, for instance the bicyclic *E*-type mimic present herein and the positively charged phosphoramidate α-DNA,[9] might increase the scopes for parallel nucleic acid recognition to an even higher level, and might lead to therapeutically interesting oligonucleotides. On the other hand, regarding the low DNA and RNA affinity found for sequence **26**, it seems unlikely that the thermal stability of parallel nucleic acid duplexes can surpass the level found for fully modified α-LNA[10,11] and β-L-LNA sequences.[11] Thereby, parallel duplexes might not ever reach the same level of thermal stability as antiparallel duplexes.

Conclusion

A bicyclic α-D-configured nucleoside has been efficiently synthesized and proved to be strongly restricted in an *E*-type conformation. By incorporation of this into α-D-configured oligodeoxynucleotides, the scopes of parallel nucleic acid duplex formation has been illuminated. The ability of α-DNA to form strong parallel duplexes with complementary DNA seems to be closely related to its strong tendency to adopt *S*-type conformations. On the other hand, we have demonstrated that the parallel duplex formed between α-DNA and complementary RNA can be slightly stabilized by mixmers with a conformationally restricted *E*-type mimic dependent on sequence constitution.

Experimental Section

General Methods. Reactions were performed under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out on glass columns using silica gel 60 (0.040−0.063 mm). NMR spectra were recorded at 200 or 300 MHz for ^{1}H NMR, 75 MHz for ^{13}C NMR, and 121.49 MHz for ^{31}P NMR. The δ values are in ppm relative to tetramethylsilane as internal standard (for ^{1}H and ^{13}C NMR) and relative to 85% H_3PO_3 as external standard (for ^{31}P NMR). Assignments of NMR spectra when given are based on 2D spectra and follow standard carbohydrate and nucleoside style; i.e., the carbon atom next to a nucleobase is assigned C-1′, etc. In nucleosides, C-1″ designates the carbon atom in the 3′-C branch; otherwise, C-1′ designates the carbon atom in the 3-C branch. Compound names given in this section for the bicyclic compounds are given according to the von Baeyer nomenclature. HRMALDI and EI mass spectra were recorded in positive-ion mode except for the oligonucleotides where these were recorded in negative-ion mode.

3,5-Di-*O*-benzyl-1,2-di-*O*-isopropylidene-3-*C*-(methanesulfonyloxymethyl)-α-D-ribofuranose (7). A solution of **6**[19] (113 mg, 0.28 mmol) in anhydrous pyridine (5 mL) was cooled to 0 °C. Methanesulfonyl chloride (44 μL, 0.56 mmol) was added dropwise at 0 °C, and the mixture was stirred at room temperature for 2 h. The reaction mixture was quenched by adding ice−water (20 mL), and the resulting mixture was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), and concentrated under reduced pressure to yield **7** (127 mg, 94%) as a clear oil which was used without further purification in the next step: R_f 0.65 (19:1 CH_2Cl_2/CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.24 (m, 10H, Ph), 5.86 (d, 1H, J_{H1-H2} = 3.6 Hz, H-1), 4.84−4.72 (m, 3H), 4.58−4.53 (m, 2H), 4.41 (s, 2H), 4.34 (m, 1H, H-4), 3.76 (m, 1H, H-5a), 3.66 (m, 1H, H-5b), 2.84 (s, 3H, SO_2CH_3), 1.61 (s, 3H, CH_3), 1.38 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.4, 137.8, 128.5, 128.4, 128.0, 127.9, 127.8, 127.7 (Ph), 113.2 ($C(CH_3)_2$), 104.1 (C-1), 83.7, 79.6, 78.7, 73.8, 69.6, 68.9, 66.9 (C-3, C-2, C-4, 2 × CH_2Ph, C-5, C-1′), 37.3 (SO_2CH_3), 26.7 (CH_3), 26.7 (CH_3); HRMALDI MS *m/z* 501.1556 ($[M + Na]^+$, $C_{24}H_{30}O_8SNa^+$ calcd 501.1553).

Methyl 3,5-Di-*O*-benzyl-3-*C*-(methanesulfonyloxymethyl)-D-ribofuranoside (8). Anhydrous CH_3OH (4 mL) was stirred at −30 °C, and acetyl chloride (0.68 mL) was added slowly. The mixture was stirred at −30 °C for 30 min and warmed to room temperature. A solution of **7** (204 mg, 0.43 mmol) in CH_2Cl_2/CH_3OH/H_2O (2:2:1 (v/v/v), 5 mL) was stirred at 0 °C, CH_3OH was added dropwise. The mixture was stirred at room temperature for 90 h, and water (5 mL) was added. The mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH (0−2%) in CH_2Cl_2) to give **8** (127 mg, 66%, α/β = 1:2.3) as a colorless viscous oil: R_f 0.50 (19:1 CH_2Cl_2/CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.25 (m, Ph), 5.00−4.44 (m), 4.13 (s), 3.7−3.59 (m), 3.49 (s, $OCH_3\alpha$), 3.39 (s, $OCH_3\beta$), 2.93 (s, $SO_2CH_3\beta$), 2.90 (s, $SO_2CH_3\alpha$); ^{13}C NMR (300 MHz, $CDCl_3$) δ 138.4, 137.5, 137.5, 137.3, 128.8, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.3, 109.0, 102.0, 82.7, 81.4, 80.5, 80.0, 77.9, 76.5, 75.2, 74.0, 73.9, 72.9, 69.9, 69.0, 68.8, 68.4, 67.3, 67.2, 66.9, 56.0, 55.8, 37.5, 37.5; HRMALDI MS *m/z* 475.1405 ($[M + Na]^+$, $C_{22}H_{28}O_8SNa^+$ calcd 475.1397).

3-*C*-Acetoxymethyl-3,5-di-*O*-benzyl-1,2-di-*O*-isopropylidene-α-D-ribofuranose (14). To a stirred solution of **6** (579 mg, 1.45 mmol) in anhydrous pyridine (5 mL) was added acetic anhydride (0.68 mL, 7.24 mmol), and the reaction mixture was stirred for 2 h at room temperature. The reaction was quenched with ice-cold water (20 mL) and extracted with CH_2Cl_2 (2 × 25 mL). The combined organic phase was washed with saturated aqueous sodium hydrogen carbonate (3 × 25 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give **14** (628 mg, 98%) as an oily residue which was used without further purification in the next step: R_f 0.70 (19:1 CH_2Cl_2/CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.24 (m, 10H, Ph), 5.83 (d, 1H, J_{H1-H2} = 3.9 Hz, H-1), 4.77, 4.65 (AB, 2H,

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J_{gem} = 10.8 Hz, 3-OCH_2Ph), 4.61−4.51 (m, 3H, H-2, 5-OCH_2-Ph), 4.39 (m, 1H, H-4), 4.35, 4.18 (AB, 2H, J_{gem} = 12.6 Hz, H-1′), 3.75 (m, 1H, H-5a), 3.60 (m, 1H, H-5b), 2.04 (s, 3H, $COCH_3$), 1.61 (s, 3H, CH_3), 1.37 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5 (CO), 138.6, 138.0, 128.4, 128.3, 127.9, 127.8, 127.7, 127.7, 127.6 (Ph), 113.0 ($C(CH_3)_2$), 104.3 (C-1), 83.4 (C-3), 80.4 (C-4), 79.3 (C-2), 73.7 (5-OCH_2Ph), 68.2 (3-OCH_2Ph), 67.8 (C-5), 63.1 (C-1′), 26.9 (CH_3), 26.8 (CH_3), 21.0 ($COCH_3$); HRMALDI MS *m/z* 465.1868 ([M + Na]$^+$, $C_{25}H_{30}O_7Na^+$ calcd 465.1883).

Methyl 3,5-Di-*O*-benzyl-3-*C*-(hydroxymethyl)-D-ribofuranoside (15). The same procedure as in the preparation of **8** was used with the ribofuranose **14** (588 mg, 1.33 mmol) affording a mixture of **15** and an unidentified material (471 mg): R_f 0.35 (19:1 CH_2Cl_2/CH_3OH); HRMALDI MS *m/z* 397.1604 ([M + Na]$^+$, $C_{21}H_{26}O_6Na^+$ calcd 397.1621).

Methyl 2-*O*-Acetyl-3-*C*-(benzoyloxymethyl)-3,5-di-*O*-benzyl-D-arabinofuranoside (18). To a stirred solution of **6** (1.661 g, 4.15 mmol) in anhydrous pyridine (6 mL) at 0 °C was added benzoyl chloride (1.204 mL, 10.38 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was concentrated to dryness under reduced pressure and coevaporated with toluene (3 × 15 mL) under reduced pressure. The residue was partitioned between ethyl acetate (15 mL) and water (9 mL). The separated organic phase was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 15 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give **16** as an oily residue. In a separate flask, anhydrous CH_3OH (33 mL) was stirred at −30 °C, and acetyl chloride (6.6 mL) was added slowly. The mixture was stirred at −30 °C for 30 min and warmed to room temperature. A solution of the oily intermediate **16** in CH_2Cl_2/ CH_3OH /H_2O (2:2:1 (v/v/v), 41.5 mL) was stirred at 0 °C, and the first solution of acetyl chloride in CH_3OH was added dropwise. The mixture was stirred at room temperature for 96 h, and water (41 mL) was added. The mixture was neutralized with saturated aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness under reduced pressure to afford **17** as an anomeric mixture (β/α = 2.7:1 on the basis of a crude ^{1}H NMR): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02−7.99 (m), 7.59−7.21 (m), 4.99−4.90 (m), 4.83−4.43 (m), 4.27−4.14 (m), 3.77−3.56 (m), 3.50 (s, OCH_3 α), 3.41 (s, OCH_3 β), 3.16−3.11 (m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.2, 166.2, 138.7, 137.7, 137.6, 133.6, 133.5, 133.4, 133.3, 130.0, 129.9, 129.8, 129.8, 128.8, 128.6, 128.6, 128.6, 128.5, 128.4, 128.2, 128.2, 128.0, 127.9, 127.8, 127.7, 127.5, 127.2, 109.3, 102.1, 83.0, 81.6, 80.8, 80.4, 80.1, 78.5, 75.5, 75.3, 74.5, 73.9, 73.8, 73.5, 71.7, 69.6, 68.9, 67.0, 66.8, 64.3, 62.6, 56.0, 55.7; HRMALDI MS *m/z* 501.1861 ([M + Na]$^+$, $C_{28}H_{30}O_7Na^+$ calcd 501.1883). The residue of crude **17** was dissolved in a mixture of anhydrous CH_2Cl_2 (45 mL) and anhydrous pyridine (9 mL). After the mixture was cooled to −30 °C, trifluoromethanesulfonic anhydride (1.47 mL, 8.90 mmol) was added dropwise. The reaction mixture was allowed to warm to 0 °C. After being stirred for an additional 30 min, the mixture was diluted with CH_2Cl_2 (100 mL), washed with a saturated aqueous solution of $NaHCO_3$ (3 × 125 mL), and dried (Na_2SO_4). After evaporation of the solvents, the residue was coevaporated with anhydrous toluene (2 × 50 mL) and dissolved in anhydrous DMF (24 mL). CsOAc (4.23 g, 22.1 mmol) and 18-crown-6 (2.120 g, 8.02 mmol) were added, and the mixture was stirred for 30 min at room temperature and then heated to 95 °C for 2 h. After cooling to room temperature the mixture was diluted with ethyl acetate (150 mL) and washed with water (2 × 100 mL) and brine (2 × 50 mL), dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH (0−0.1%) in CH_2Cl_2) to give **18** as a white foam (973 mg, 46% over four steps, α/β = 1.1:1): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98−7.93 (m), 7.60−7.15 (m), 5.56 (s), 5.30 (d, J = 4.8 Hz), 5.21 (d, J = 4.8 Hz), 4.92−4.87 (m), 4.77−4.38 (m), 3.87−3.62 (m), 3.44 (s, OCH_3 α), 3.40 (s, OCH_3 β), 2.03 (s, $COCH_3$ β), 2.01 (s, $COCH_3$ α); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.6, 169.4, 166.2, 166.2, 138.1, 137.9, 137.8, 137.8, 133.4, 133.3, 130.0, 130.0, 129.9, 129.9, 129.8, 129.7, 128.7, 128.6, 128.6, 128.5, 128.5, 128.4, 128.3, 128.0, 128.0, 127.9, 127.9, 127.9, 127.8, 127.6, 127.5, 127.5, 107.2 (C-1 α), 102.1 (C-1 β), 85.3, 84.2, 82.6, 81.3, 81.3, 73.7, 69.9, 68.7, 66.84, 66.2, 60.7, 60.1, 56.6, 55.4, 20.9, 20.8; HRMALDI MS *m/z* 543.1973 ([M + Na]$^+$, $C_{30}H_{32}O_8Na^+$ calcd 543.1989).

***N*1-(2-*O*-Acetyl-3-*C*-(benzoyloxymethyl)-3,5-di-*O*-benzyl-α-D-arabinofuranosyl)thymine (19).** To a stirred solution of **18** (470 mg, 0.90 mmol) and thymine (228 mg, 1.81 mmol) in anhydrous CH_3CN (15 mL) was added *N,O*-bis(trimethylsilyl)acetamide (1.8 mL, 7.23 mmol). The reaction mixture was stirred at reflux for 30 min. After cooling to 0 °C, trimethylsilyl triflate (0.82 mL, 4.52 mmol) was added dropwise, and the solution was stirred for 24 h at 70 °C. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (50 mL) and washed with saturated aqueous $NaHCO_3$ solution (3 × 30 mL). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH (0−3.0%) in CH_2Cl_2) to give nucleoside **19** (380 mg, 68%) as a white foam: R_f 0.50 (9:1 CH_2Cl_2/CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (s, 1H, NH), 8.01−7.98 (m, 2H, Ph), 7.61−7.25 (m, 14H, H-6, Ph), 6.14 (d, 1H, $J_{H1'-H2'}$ = 2.4 Hz, H-1′), 5.48 (d, 1H, $J_{H1'-H2'}$ = 2.4 Hz, H-2′), 4.88−4.54 (m, 7H), 3.77−3.66 (m, 2H), 1.95 (s, 3H, $COCH_3$), 1.49 (d, 3H, J = 1.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.9 ($COCH_3$), 166.1 (*C*OPh), 163.7 (C-4), 150.4 (C-2), 137.2 (Ph), 136.5 (C-6), 136.2, 133.6, 129.8, 129.3, 128.8, 128.7, 128.7, 128.4, 128.3, 128.3, 128.2 (Ph), 111.2 (C-5), 90.1, 86.1, 83.3, 79.8, 74.0, 68.4, 66.3, 59.4 (C-1′, C-2′, C-3′, C-4′, C-5′, C-1″, 2 × CH_2Ph), 20.6 ($COCH_3$), 12.2 (CH_3); HRMALDI MS *m/z* 637.2150 ([M + Na]$^+$, $C_{34}H_{34}N_2O_9Na^+$ calcd 637.2156). Anal. Calcd for $C_{34}H_{34}N_2O_9$: C, 66.44; H, 5.57; N, 4.56. Found: C, 66.28; H, 5.52; N, 4.59.

***N*1-(3,5-Di-*O*-benzyl-3-*C*-(hydroxymethyl)-α-D-arabinofuranosyl)thymine (11).** Sodium methoxide (134 mg, 2.48 mmol) was added to a solution of **19** (380 mg, 0.62 mmol) in anhydrous CH_3OH (5 mL), and the reaction mixture was stirred for 2 h at room temperature. Excess sodium methoxide was neutralized with dilute aqueous hydrochloric acid. The mixture was extracted with CH_2Cl_2 (2 × 20 mL), and the combined extract was washed with saturated aqueous $NaHCO_3$ solution (3 × 15 mL), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH (0−3.25%) in CH_2Cl_2) to give nucleoside **11** as a white foam (260 mg, 90%): R_f 0.40 (9:1 CH_2Cl_2/CH_3OH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.14 (s, 1H, NH), 7.40−7.16 (m, 11H, H-6, Ph), 5.86 (s, 1H, H-1′), 5.29 (d, 1H, $J_{H2'-OH}$ = 6.3 Hz, OH), 4.69−4.42 (m, 6H), 4.16−4.11 (m, 2H), 3.88 (m, 1H, H-5′a), 3.74 (m, 1H, H-5′b), 3.00 (m, 1H, OH), 1.51 (d, 3H, J = 1.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.5 (C-4), 151.0 (C-2), 137.1, 136.9, 136.6, 128.8, 128.7, 128.4, 128.2, 128.1 (C-6, Ph), 109.8 (C-5), 94.4, 88.0, 86.6, 79.3, 74.2, 69.7, 66.0, 58.3 (C-1′, C-2′, C-3′, C-4′, C-5′, C-1″, 2 × CH_2Ph), 12.3 (CH_3); HRMALDI MS *m/z* 491.1766 ([M + Na]$^+$, $C_{25}H_{28}N_2O_7Na^+$ calcd 491.1788). Anal. Calcd for $C_{25}H_{28}N_2O_7 \cdot {}^1/_4H_2O$: C, 63.48; H, 6.07; N, 5.92. Found: C, 63.35; H, 5.94; N, 5.86

***N*1-(3,5-Di-*O*-benzyl-3-*C*-(methanesulfonyloxymethyl)-α-D-arabinofuranosyl)thymine (12).** A solution of **11** (373 mg, 0.82 mmol) in anhydrous pyridine (3 mL) was cooled to −40 °C. Methanesulfonyl chloride (64 μL, 0.82 mmol) was added dropwise, and the mixture was stirred at −40 °C for 2 h and then at room temperature for 1 h. The reaction mixture was cooled to 0 °C and water (25 mL) was added followed by a saturated aqueous solution of $NaHCO_3$ (25 mL). The resulting mixture was extracted with CH_2Cl_2 (3 × 25 mL), and the organic phase was dried (Na_2SO_4). The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH (0−

3.0%) in CH_2Cl_2) to give nucleoside **12** as a white foam (364 mg, 84%): R_f 0.45 (9:1 CH_2Cl_2/CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 9.70 (br s, 1H, NH), 7.42−7.18 (m, 11H, H-6, Ph), 5.93 (s, 1H, H-1′), 5.31 (m, 1H, OH), 4.74−4.40 (m, 8H, H-4′, 2 × CH_2Ph, 2 × H-1″, H-2′), 3.85−3.74 (m, 2H, 2 × H-5′), 2.94 (s, 3H, SO_2CH_3), 1.45 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.4 (C-4), 150.6 (C-2), 136.9, 136.4, 136.3, 128.9, 128.8, 128.5, 128.4, 128.2 (C-6, Ph), 109.9 (C-5), 94.1, 86.1, 85.3, 79.3, 74.2, 69.2, 66.2, 64.7 (C-1′, C-2′, C-3′, C-4′, C-5′, C-1″, 2 × CH_2-Ph), 37.4 (SO_2CH_3), 12.2 (CH_3); HRMALDI MS: *m/z* 569.1577 ($[M + Na]^+$, $C_{26}H_{30}N_2O_9SNa^+$ calcd 569.1564).

(1*R*,2*R*,4*S*,5*S*)-1-(Benzyloxy)-2-(benzyloxymethyl)-4-(thymin-1-yl)-3,6-dioxabicyclo[3.2.0]heptane (13). A solution of nucleoside **12** (364 mg, 0.67 mmol) in anhydrous dioxane (5 mL) was cooled to 10 °C. A 60% oily dispersion of NaH (67 mg, 1.67 mmol) was added in one portion. The reaction mixture was stirred at 50 °C for 2 h and then quenched with saturated aqueous NH_4Cl solution (20 mL). The reaction mixture was extracted with CH_2Cl_2 (4 × 15 mL). The combined organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH 0−1.5% in CH_2Cl_2) to afford nucleoside **13** (293 mg, 98%) as a white foam: R_f 0.45 (9:1 CH_2Cl_2/CH_3OH); ^1H NMR (300 MHz, $CDCl_3$) δ 8.98 (s, 1H, NH), 7.34−7.25 (m, 10H, Ph), 7.03 (d, 1H, J = 1.2 Hz, H-6), 5.88 (s, 1H, H-1′), 5.50 (s, 1H, H-2′), 4.86−4.58 (m, 6H, 2 × H-1″, 2 × CH_2Ph), 4.52 (m, 1H, H-4′), 3.86 (m, 1H, H-5′a), 3.74 (m, 1H, H-5′b), 1.86 (d, 3H, J = 1.2 Hz, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.8 (C-4), 150.6 (C-2), 137.5, 137.4 (Ph), 137.2 (C-6), 128.7, 128.6, 128.2, 128.1, 128.0, 127.5 (Ph), 111.4 (C-5), 91.1 (C-1′), 91.1 (C-2′), 86.0 (C-3′), 84.4 (C-4′), 73.9, 73.3, 68.9, 68.7 (2 × *C*H_2Ph, C-5′, C-1″), 12.6 (CH_3); HRMALDI MS *m/z* 473.1666 ($[M + Na]^+$, $C_{25}H_{26}N_2O_6Na^+$ calcd 473.1683). Anal. Calcd for $C_{25}H_{26}N_2O_6$: C, 66.65; H, 5.81; N, 6.21. Found: C, 66.50; H, 5.86; N, 6.10.

(1*R*,2*R*,4*S*,5*S*)-1-Hydroxy-2-(hydroxymethyl)-4-(thymin-1-yl)-3,6-dioxabicyclo[3.2.0]heptane (4). To a stirred solution of nucleoside **13** (280 mg, 0.62 mmol) in ethanol (5 mL) was added 20% palladium hydroxide over carbon (140 mg). The mixture was degassed several times with argon and placed under a hydrogen atmosphere. The reaction mixture was stirred at room temperature for 6 h and then filtered through Celite. The filtrate was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH (0−4.0%) in CH_2Cl_2) to give nucleoside **4** as a white foam (165 mg, 98%): R_f 0.25 (17:3 CH_2Cl_2/CH_3OH); ^1H NMR (300 MHz, CD_3OD) δ 7.36 (d, 1H, J = 1.2 Hz, H-6), 5.73 (s, 1H, H-1′), 5.27 (s, 1H, H-2′), 4.80 (m, 1H, H-1″a), 4.58 (m, 1H, H-1″b), 4.15 (m, 1H, H-4′), 3.91 (m, 1H, H-5′a), 3.77 (m, 1H, H-5′b), 1.88 (d, 3H, J = 1.2 Hz, CH_3); ^{13}C NMR (75 MHz, CD_3OD) δ 166.4 (C-4), 152.7 (C-2), 139.7 (C-6), 111.6 (C-5), 95.4 (C-2′), 92.7 (C-1′), 87.3 (C-4′), 81.3 (C-3′), 77.6 (C-1″), 61.9 (C-5′), 12.4 (CH_3); HRMALDI MS *m/z* 293.07470 ($[M + Na]^+$, $C_{11}H_{14}N_2O_6Na^+$ calcd 293.07441).

(1*R*,2*R*,4*S*,5*S*)-1-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-2-(4,4′-dimethoxytrityloxymethyl)-4-(thymin-1-yl)-3,6-dioxabicyclo[3.2.0]heptane (21). To a solution of diol **4** (160 mg, 0.593 mmol) in anhydrous pyridine (4 mL) was added 4,4′-dimethoxytrityl chloride (502 mg, 1.482 mmol), and the mixture was stirred at room temperature for 18 h. The reaction was quenched with CH_3OH (1.5 mL), and the mixture was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: gradient of CH_3OH (0−2.5%) in a solution of 0.5% pyridine in CH_2Cl_2) to give **20** as a white foam: R_f = 0.80 (17:3 CH_2Cl_2/CH_3OH); ^1H NMR (200 MHz, CD_3OD) δ 9.21 (s, 1H, NH), 7.47−7.15 (m, 9H, Ar), 7.02 (d, 1H, J = 1.0 Hz, H-6), 6.82 (d, 4H, J = 8.0 Hz), 5.34 (s, 1H, H-1′), 5.24 (s, 1H, H-2′), 4.60−4.36 (m, 3H, H-1″, H-4′), 3.78 (s, 6H, OCH_3), 3.52 (m, 1H, H-5′a), 3.22 (m, 1H, H-5′b), 1.95 (d, 3H, J = 1.0 Hz, CH_3). Intermediate **20** was dissolved in anhydrous CH_2Cl_2 (5 mL). *N,N*-Diisopropylethylamine (0.32 mL, 1.89 mmol) followed by 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.21 mL, 0.94 mmol) were added, and the mixture was stirred at room temperature for 23 h. The reaction was quenched with CH_3OH (3 mL), and the mixture was dissolved in ethyl acetate (70 mL), washed with saturated aqueous $NaHCO_3$ (3 × 50 mL) and brine (3 × 50 mL), dried (Na_2SO_4), and was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: gradient of ethyl acetate (0−60%) in a solution of 0.5% pyridine in *n*-hexane) to give **21** as a white foam (286 mg, 79%): ^{31}P NMR ($CDCl_3$) δ 146.31, 145.95; HRMALDI MS: *m/z* 795.3113 ($[M + Na]^+$, $C_{41}H_{49}N_4O_9PNa^+$ calcd 795.3129).

Synthesis of Oligodeoxynuclotides: Oligonucleotide synthesis was carried out by using an automated DNA synthesizer following the phosphoramidite approach. Synthesis of α-oligonucleotides **22**−**30** was performed on a 0.2 μmol scale by using α-thymidine 2-cyanoethyl phosphoramidite,[36] N-benzoyl-protected α-5-methylcytosine 2-cyanoethyl phosphoramidite,[37] as well as compound **21**. The synthesis followed the regular protocol for DNA synthesizer and a universal CPG support (Biogenex/Glen Research) was employed. However, for compound **21**, a prolonged coupling time of 10 min was used. Coupling yields for all 2-cyanoethyl phosphoramidites were >98%. The 5′-*O*-DMT-*ON* oligonucleotides were removed from the universal solid support by treatment with 2% LiCl in concentrated ammonia at 55 °C for 20 h, which also removed the protecting groups. Subsequent purification using reversed-phase HPLC (column xterra C18 (10 μm) 7.8 × 300 mm, with a gradient of buffers A and B (A, 90% 0.1 M NH_4HCO_3 + 10% CH_3CN; B, 25% 0.1 M NH_4HCO_3 + 75% CH_3CN), 1 mL/min.), 5′-*O*-detritylation by acetic acid, and another HPLC purification (same conditions) afforded the > 90% pure oligonucleotides. MALDI-MS $[M - H]^-$ gave the following results (found/calcd): **22** (4196.9/4196.8); **23** (4222.5/4224.8); **24** (4306.7/4308.8); **25** (2976.4/2980.0); **26** (3259.6/3260.1); **27** (2975.3/2976.0); **28** (3003.6/3004.0); **29** (3058.9/3060.0); **30** (3141.8/3144.1).

Thermal Denaturation Experiments. The thermal denaturation studies were performed in a medium salt buffer containing Na_2HPO_4 (10 mM), NaCl (100 mM) and EDTA (0.1 mM), pH 7.0 with 1 μM concentrations of the two complementary sequences. The extinction coefficients were calculated assuming the extinction coefficients for all thymine nucleotides to be identical. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was increased linearly from 10 to 65 °C at a rate of 0.5 °C/min by means of a 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.

Quantum Mechanical Calculations. All ab initio quantum mechanical calculations were carried out using the Gaussian 98 program.[49] To determine the potential energy of pseudorotation, sugar torsion angles ν_2 and ν_4 were calculated using the theory of Altona and Sundaralingam with a maximal puckering amplitude of 38°.[31] For each point along the pseudorotation pathway, a full geometry optimisation (HF/6-31G) was carried out while maintaining the desired ν_2 and ν_4 angles. To ensure that no hydrogen bonds were formed

(49) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11.3; Gaussian, Inc.: Pittsburgh, PA, 1998.

between the 3′-hydroxy group and the nucleobase, the ϵ torsion angle was constrained to −75°.[47] The β, γ, and χ angles were not constrained but their initial values before optimization were taken from the NMR structure of Aramini et al.[47] In the optimized structures there is no steric interactions between the 5′-hydroxy group, the sugar and the nucleobase. Single point energies were determined using second-order Møller−Plesset theory (MP2) with the cc-pVTZ basis set. An unrestrained geometry optimization using MP2/6-31G* was also performed.

Karplus Equation. A Karplus relationship correlating $J_{H1'-H2'}$ and the ν_1 torsion angle was constructed using a state-of-the-art Karplus equation (eq 1) for nucleosides and nucleotides developed by Altona and co-workers,[32−34] where the electronegativity of the HCCH-fragment substituents is accounted for in the coefficients C_m and S_n.

$$J_{HH}(\theta) = \sum_{m=0}^{3} C_M \cos(m\theta) + \sum_{=1}^{3} S_n(n\theta) \qquad (1)$$

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Supporting Information Available: Melting curves supporting the thermal denaturation studies. HPLC profiles for oligonucleotides. Full coverage of the efforts toward **4** following route A, Figure 2 including experimental details for new compounds **S1**−**S5**, **S7**, **S8**, and **S10**−**S15**. Experimental details for the preparation of **6**. Experimental details for the alternative preparation of **12** from **8**. ^{1}H and ^{13}C NMR spectra for compound **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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