A Novel Class of Oligonucleotide Analogues Containing 2′-*O*,3′-*C*-Linked [3.2.0]Bicycloarabinonucleoside Monomers: Synthesis, Thermal Affinity Studies, and Molecular Modeling

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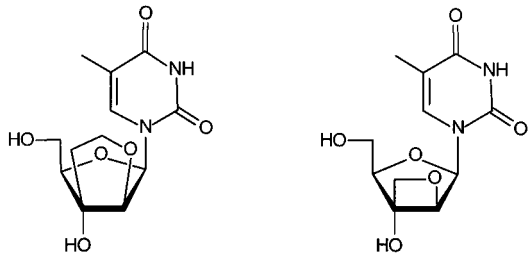
Abstract: Oligonucleotide analogues containing a novel 2′-*O*,3′-*C*-linked [3.2.0]bicyclonucleoside have been efficiently synthesized. Enhanced thermal stabilities of duplexes toward both RNA and DNA are reported for a 14-mer oligothymidylate containing 13 modifications and for a nonamer mixed sequence containing three modifications. These results and the results from molecular modeling reveal that strong conformational restriction of a monomer can be important for favorable duplex formation though the fixed conformation of the pentofuranose ring deviates from a North or South conformation.

Introduction

Despite the huge number of chemically modified oligonucleotides (ONs) that have been synthesized during the last years,[1] an ideal modification has not yet been obtained keeping, e.g., the requirements for potential pharmaceutical applications in mind. When designing novel ON analogues, we have identified the following chemical characteristics as desired: (a) absence of additional functional groups (which require protection during oligomerization), (b) efficient automated oligomerization (also in combination with unmodified DNA/RNA monomers), (c) good aqueous solubility of oligomers, (d) stability toward nucleases, and (e) enhanced binding affinity and specificity toward complementary DNA and/or RNA (also for partly modified mixed sequences). We consider ONs consisting of bicyclopentofuranose nucleotides as prime candidates for such optimized analogues.

Interesting results have been achieved for conformationally restricted ON analogues having a potential entropic advantage during duplex formation.[2] Stimulated by this and by our recent results[3] on ONs containing [3.3.0]bicyclo nucleoside **1** (Figure 1) exhibiting strong RNA-selective recognition in an almost fully modified ON, we have synthesized the novel [3.2.0]bicyclo nucleoside **6** (Figure 1) and evaluated its influence on the properties of ONs.



1 **6**

Figure 1.

Results and Discussion

The bicyclic nucleoside **6** was synthesized in 12 steps from the known ulose **2**[4] in an overall yield of 8.5% (Scheme 1). The first step was a stereoselective Grignard addition of a vinyl group followed by desilylation and benzylation affording furanose **3** in 68% yield. Acetolysis, acetylation and coupling with thymine using the silyl Hilbert-Johnson/Birkofer method as modified by Vorbrüggen et al.[5] yielded after deacetylation the nucleoside **4** in 70% yield from **3**. The configuration at the 2′-carbon atom was inverted using an anhydro approach[6] by mesylation followed by treatment with aqueous base to give the desired *arabino*-configurated nucleoside. The double bond was oxidatively cleaved affording the 3′-*C*-hydroxymethyl nucleoside **5** in 22% yield from **4** after reduction. The primary hydroxy group of **5** was selectively mesylated, and the residue was treated with sodium hydride followed by debenzylation thus affording (1*R*,2*R*,4*R*,5*S*)-1-hydroxy-2-hydroxymethyl-4-(thymin-1-yl)-3,6-dioxabicyclo[3.2.0]heptane **6** in 80% yield from **5**. The structure of compound **6** was verified by NMR experiments.

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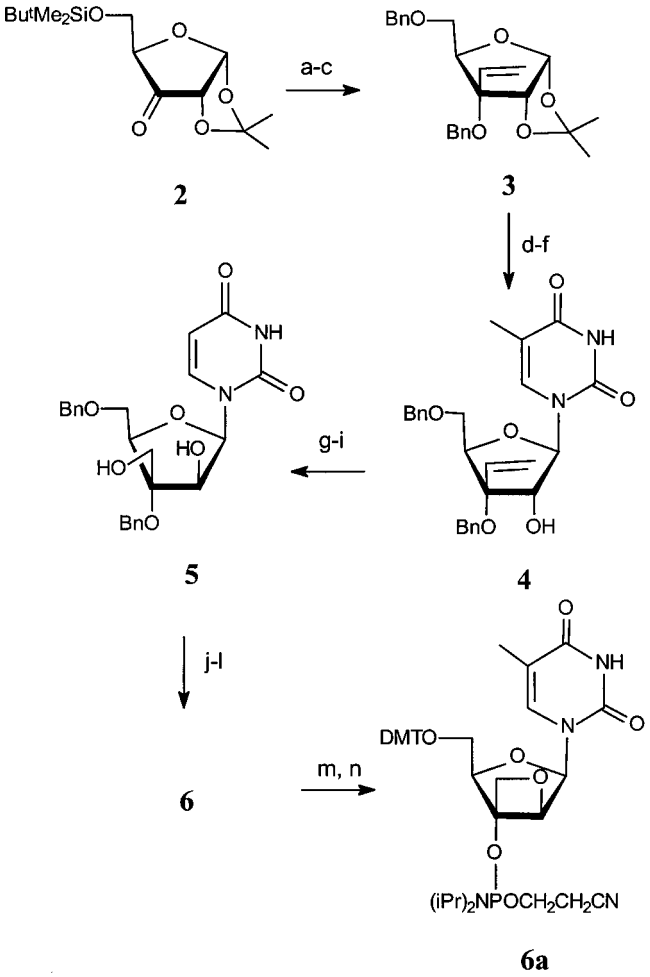
Scheme 1[a]



[a] Key: (a) Vinylmagensium bromide, Et_2O, THF; (b) Bu_4NF, THF (75% for two steps, **2a**); (c) BnBr, NaH, DMF (91%, **3**); (d) 80% AcOH, then Ac_2O, pyridine (87%, **3a**); (e) thymine, *N,O*-bis(trimethylsilyl)acetamide, CH_3CN, $Me_3SiOSO_2CF_3$ (83%, **3b**); (f) MeONa, MeOH (97%, **4**); (g) $MeSO_2Cl$, pyridine (84%, **4a**); (h) NaOH, EtOH, H_2O (74%, **4b**); (i) $NaIO_4$, catalytic OsO_4, THF, H_2O, then $NaBH_4$, THF, H_2O (36%, **5**); (j) $MeSO_2Cl$, pyridine; (k) NaH, DMF (93% for two steps, **5a**); (l) H_2, $Pd(OH)_2$/C, EtOH (86%, **6**); (m) 4,4′-dimethoxytrityl chloride (DMTCl), pyridine; (n) $NC(CH_2)_2OP(Cl)N(^iPr)_2$, CH_2Cl_2 (61% for two steps, **6a**).

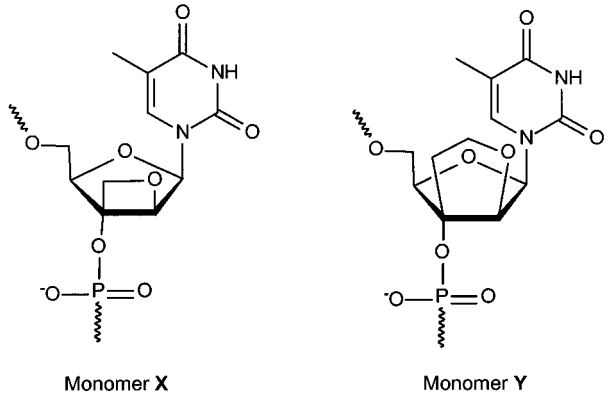
Especially, the mutual NOE effects between H-5′ and H-1″, H-1′ and H-2′, and H-1′ and H-4′ (conventional nucleoside numbering used) were indicative of the assigned β-D-*arabino* configuration. The novel bicyclic nucleoside **6** was converted to the 5′-*O*-4,4′-dimethoxytrityl-protected derivative and eventually transformed into the corresponding 3′-*O*-phosphoramidite building block **6a** suitable for automated incorporation[7] of the bicyclic monomer **X** into ONs.

Seven modified oligonucleotides containing monomer **X** were synthesized[8] and their thermal stability was evaluated[3] against that of complementary single-stranded DNA and RNA (Table 1). To allow comparison, the same oligothymidylate sequences as used in the evaluation of the bicyclic monomer **Y** derived from nucleoside **1**[3] were synthesized. Stepwise coupling yield for the phosphoramidite **6a** was approximately 95% (12 min coupling) compared to >99% for unmodified deoxynucleoside phosphoramidites (2 min coupling). After cleavage from the solid support, deprotection, and reversed-phase purification (which includes 5′-end detritylation), the purity (>90%, capillary gel electrophoresis) and the composition (MALDI-MS) of all modified ONs were verified. Using a qualitative UV method described earlier,[9] the stability of 5′-$\mathbf{X}_{13}$T toward snake venom phosphodiesterase (a 3′-exonuclease) was evaluated. Promisingly, no degradation of 5′-$\mathbf{X}_{13}$T was observed during the 60 min monitored, whereas the unmodified reference T_{14} was completely degraded within 10 min.

Table 1. Melting Experiments of Modified Oligonucleotides[a]

	complementary ssDNA		complementary ssRNA	
oligonucleotide	T_m/°C	ΔT_m/°C	T_m/°C	ΔT_m/°C
5′-T_{14}	36.0	ref	34.0	ref
5′-$T_7\mathbf{X}T_6$	36.0	0	33.5	−0.5
5′-$T_6\mathbf{X}_2T_6$	34.5	−1.5	33.0	−1.0
5′-$T_6\mathbf{X}T\mathbf{X}T_5$	35.5	−0.5	32.5	−1.5
5′-$T_5\mathbf{X}_4T_5$	31.5	−4.5	37.0	+3.0
5′-$T_3(T\mathbf{X})_4T_3$	35.5	−0.5	31.5	−2.5
5′-$\mathbf{X}_{13}$T	58.0	+23.0	49.0	+15.0
5′-GTGATATGC	33.5	ref	30.5	ref
5′-G**Y**GA**Y**A**Y**GC	26.0	−7.5	26.5	−4.0
5′-G**X**GA**X**A**X**GC	34.5	+1.0	34.5	+4.0

[a] Measured at 260 nm in medium salt buffer: 1 mM EDTA, 10 mM Na_3PO_4, 140 mM NaCl, pH 7.2. Concentration of each strand 2.5 μM; T = thymidine monomer; **X** = monomer derived from nucleoside **6**; **Y** = monomer derived from nucleoside **1**; G = 2′-deoxyguanosine monomer; A = 2′-deoxyadenosine monomer; C = 2′-deoxycytidine monomer; T_m = melting temperature determined as the maximum of the first derivative of the absorbance vs temperature curve; ΔT_m = change in T_m compared to the unmodified reference (ref).



The modified ONs (and unmodified reference strands) were mixed in a 1:1 ratio to complementary DNA or RNA strands assuming identical extinction coefficients of the modified and unmodified strands. The melting temperatures (T_m values) were determined as the maxima of the first derivatives of the absorbance versus temperature curves (Table 1). The thermodynamic data for formation of the duplexes 5′-$\mathbf{X}_{13}$T:dA_{14} and 5′-$\mathbf{X}_{13}$T:rA_{14} were determined from the first derivatives of the melting curves assuming a two-state model for helix−coil transition.[10] The calculated data (with the data for the corresponding reference duplex T_{14}:dA_{14} in parentheses) for the duplex 5′-$\mathbf{X}_{13}$T:dA_{14} are $\Delta H = -410$ kJ/mol (−398 kJ/mol) and $\Delta S = -1129$ J/mol K (−1170 J/mol K) leading to $\Delta G_{310} = -60$ kJ/mol (−35 kJ/mol), and for the duplex 5′-$\mathbf{X}_{13}$T:rA_{14} (with the data for the corresponding reference duplex T_{14}:rA_{14} in parentheses) $\Delta H = -362$ kJ/mol (−354 kJ/mol) and $\Delta S = -1013$ J/mol K (−1039 J/mol K) leading to $\Delta G_{310} = -48$ kJ/mol (−31 kJ/mol). These data indicate that the duplexes

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involving 5′-$\mathbf{X}_{13}$T are thermodynamically more stable than the unmodified reference duplexes. Apparently, this stabilization is of both enthalpic and entropic origin (gains in ΔH and diminished decreases in ΔS). The latter contribution can be explained by the preorganized structure of the bicyclopentofuranose rings in 5′-$\mathbf{X}_{13}$T. The background for the apparent enthalpic advantage is presently unclear, but solvatization effects or increased base-stacking are possible explanations. Though ΔG_{310} gives a more valid measure of the thermodynamic stability of duplexes than the melting temperature T_m, it has been shown that the experimental errors are larger for the former.[11] Consequently, we have in the remainder of this report used T_m values to estimate of the stability of the duplexes. Incorporation of **X** 1−4 times in 14-mer oligothymidylates induced decreases in the melting temperatures (T_m values) against both complementary DNA and RNA (0−4 °C decrease), the only exception being the ON 5′-$T_5\mathbf{X}_4T_5$ showing an increase of 3 °C in T_m against RNA. All T_m values were increased compared to the values obtained with monomer **Y**.[3] For 5′-$\mathbf{X}_{13}$T, significant increases in thermal stability of duplexes toward both DNA and RNA were observed (+23.0 and +15.0 °C, respectively), contrary to the results obtained earlier for 5′-$\mathbf{Y}_{13}$T where stabilization (+13 °C) only of the duplex toward RNA was observed. The effect of the novel bicyclic monomer **X** as well as **Y** were likewise evaluated in mixed sequence nonamers containing three modifications. As depicted in the table, increased T_m values resulted with monomer **X**, more so for the duplex toward the RNA complement (+4 °C) than for the duplex toward the DNA complement (+1 °C), whereas decreased T_m values were obtained with monomer **Y**.

The data show that the modified ONs containing monomer **X** exhibit neither DNA- nor RNA-selective binding. Actually, an effect like the significant increased T_m values seen for 5′-$\mathbf{X}_{13}$T toward *both DNA and RNA* has only be demonstrated earlier for few ON analogues, e.g., 2′-fluorophosphoramidates[12a] and tricyclo-DNA.[12b] The positive effects on the thermal stability of duplexes involving the mixed sequence are noteworthy not the least because they demonstrate that the monomer **X** is able to improve the thermal affinity in a context of unmodified deoxynucleotides.

In an attempt to understand the results depicted in Table 1, nucleosides **1** and **6** were evaluated using molecular modeling. The dynamic equilibrium of the furanose ring can be described by a two-state South/North model, with energy minima near pseudorotation angles of $P = 18°$ (C3′-endo) for the North-conformer and $P = 162°$ (C2′-endo) for the South conformer.[13] For duplexes of the A-type, the nucleotides are normally found in a North conformation, while duplexes of the B-type normally contain nucleotides in a South conformation.[14] Care should be taken, however, when describing nucleoside analogues as, e.g., bicyclo modifications can strain the furanose ring thus changing the positions of the energy minima rendering the South/North model useless. ^{1}H NMR spectra of both **1** and **6** were recorded at 500 MHz in the temperature range of −50 to +50 °C in steps of 20 °C. Neither of the two nucleosides showed variation in the $^3J_{HH}$ coupling constants in the bicyclic carbohydrate moieties within this temperature range. This indicates strongly that both bicyclic ring systems only exist in one conformation. If either of the nucleosides were in a fast equilibrium between two conformations, the relative populations of these conformations would be changed in the temperature interval investigated resulting in a change in $^3J_{HH}$ coupling constants. Therefore, all molecular modeling was performed under the assumption of a single preferred conformer for both nucleosides **1** and **6**. Details of model building and refinement protocols and molecular modeling are given in the Experimental Section. Molecular modeling revealed that the conformation of nucleosides **1** and **6** both are locked, although **1** apparently has a slight degree of conformational freedom. The preferred furanose conformation of **1** has a pseudorotation angle $P = 129°$ corresponding to a C1′-exo conformation, whereas P for **6** was found to be 94°, indicative of an O4′-endo conformation. From a structural point of view especially the conformation found for nucleoside **6** is interesting. For an unmodified nucleoside, a pseudorotation angle of $P = 94°$ corresponds to a "high-energy" conformation,[13a] and it is therefore indicated that the four-membered ring strains the furanose ring thus changing the energy minima. Thus it is possible to engineer nucleoside analogues with unusual conformational preference of the furanose ring. The modeling studies partly explain the observed changes in T_m values upon binding of the modified ONs to complementary DNA or RNA. For the ON 5′-$\mathbf{X}_{13}$T, we anticipate that the modification **X** is able to force the duplexes into forms that are different from the A-form typical of RNA−RNA and RNA−DNA duplexes and the B-form adopted by most DNA−DNA duplexes. Thus, we ascribe the increases in thermal stability to the structural preorganization of the **X** monomers (and 5′-$\mathbf{X}_{13}$T). It appears from the melting results that incorporation of several **X** monomers is needed to induce a favorable regular structure of the duplexes, i.e., the three **X** modifications are sufficient to stabilize the duplexes involving the nonamer. Contrary to the dual DNA and RNA recognition potential revealed here for 5′-$\mathbf{X}_{13}$T, the corresponding ON containing the modification **Y** (derived from **1**) showed no binding to dA_{14}.[3] This difference may be caused by the different conformations of nucleosides **1** and **6** or can be a steric effect caused by the more bulky 2′,3′-linked five-membered ring in nucleoside **1**.

Conclusion

With reference to the points a−e listed in the Introduction as desired for a chemically modified ON analogue, the results described in this report underline the potential of conformationally restricted bicyclopentofuranose nucleotides as monomers in ON analogues. Especially, the recognition of both complementary DNA and RNA with enhanced thermal stabilities described herein for ONs containing monomer **X** suggests that this analogue should be further examined. The results also indicate that strong conformational restriction of a monomer can be important for favorable duplex formation even if the preorganization results in pentofuranose conformations deviating from the ones found for the unmodified nucleotides in natural duplexes.

Experimental Section

General. All reagents were obtained from commercial suppliers and were used without further purification. After any organic phase was dried with Na_2SO_4, filtration was performed. The silica gel (0.040−0.063 mm) used for column chromatography was purchased

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from Merck. NMR spectra were recorded at 300 or 250 MHz for ^{1}H NMR, 62.9 MHz for ^{13}C NMR, and 202.33 MHz for ^{31}P NMR. The δ values are in ppm relative to tetramethylsilane as internal standard (^{1}H and ^{13}C NMR) and relative to 85% H_3PO_4 as external standard (^{31}P NMR). Assignments of NMR peaks are given according to standard nucleoside nomenclature and are based on two-dimensional NMR experiments. Coupling constants (*J*) are given in hertz.

1,2-*O*-Isopropylidene-3-*C*-vinyl-α-D-ribofuranose (2a). A solution of ulose **2**[4] (6.05 g, 0.020 mol) in anhydrous THF (250 cm^3) was stirred at 0 °C, and a 1 M solution of vinylmagnesium bromide in ether (44 cm^3, 44 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 2 h, whereupon saturated aqueous ammonium chloride (200 cm^3) was added and extraction was performed using dichloromethane (3 × 300 cm^3). The combined extract was washed with brine (3 × 250 cm^3) and dried (Na_2SO_4). The solvent was removed, and the residue was redissolved in anhydrous THF (225 cm^3). To this mixture was added a 1 M solution of tetrabutylammonium fluoride in THF (22 cm^3, 22 mmol), stirring at room temperature was continued for 20 min whereupon the mixture was evaporated under reduced pressure. The residue was dissolved in dichloromethane (500 cm^3) and washed with a saturated solution of sodium hydrogencarbonate (2 × 200 cm^3). The aqueous phase was extracted using continuous extraction for 12 h, and the combined extract was dried (Na_2SO_4) and evaporated. The residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give furanose **2a** as a white solid material (3.24 g, 75%): δ_H ($CDCl_3$) 5.84 (1H, d, *J* 3.7, 1-H), 5.74 (1H, dd, *J* 11.0, 17.2, 1′-H), 5.52 (1H, dd, *J* 1.6, 17.1, 2′-H$_a$), 5.29 (1H, dd, *J* 1.3, 11.0, 2′-H$_b$), 4.21 (1H, d, *J* 3.7, 2-H), 3.98 (1H, t, *J* 5.7, 4-H), 3.68−3.64 (2H, m, 5-H$_a$, 5-H$_b$), 2.88 (1H, s, 3-OH), 1.99 (1H, t, *J* 6.3, 5-OH), 1.60 (3H, s, CH_3), 1.35 (3H, s, CH_3): δ_C ($CDCl_3$) 133.6 (C-1′), 116.2 (C-2′), 113.0 ($C(CH_3)_2$), 103.8 (C-1), 83.4, 82.4 (C-4, C-2), 79.6 (C-3), 61.3 (C-5), 26.5, 26.4 (CH_3).

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-3-*C*-vinyl-α-D-ribofuranose (3). A 60% suspension of sodium hydride (w/w, 1.78 g, 44.5 mmol) in anhydrous DMF (50 cm^3) was stirred at 0 °C, and a solution of furanose **2a** (3.20 g, 14.8 mmol) in anhydrous DMF (35 cm^3) was added dropwise over 30 min. The mixture was stirred at 50 °C for 1 h and subsequently cooled to 0 °C. A solution of benzyl bromide (5.3 mL, 44.5 mmol) in anhydrous DMF (5.3 cm^3) was added dropwise, and the mixture was stirred at room temperature for 20 h. The reaction mixture was evaporated and redissolved in dichloromethane (300 cm^3), washed with saturated aqueous sodium hydrogencarbonate (3 × 200 cm^3), and dried (Na_2SO_4). The solvents were removed under reduced pressure, and the residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (9:1, v/v) as eluent to give furanose **3** as a white solid material (5.36 g, 91%): δ_H ($CDCl_3$) 7.40−7.26 (10H, m, Bn), 5.90 (1H, d, *J* 3.6, 1-H), 5.72 (1H, dd, *J* 11.1, 17.9, 1′-H), 5.41 (1H, dd, *J* 0.7, 11.1, 2′-H$_a$), 5.30 (1H, dd, *J* 0.5, 17.8, 2′-H$_b$), 4.70−4.45 (6H, m, Bn, 2-H, 4-H), 3.69 (1H, dd, *J* 2.6, 10.8, 5-H$_a$), 3.50 (1H, dd, *J* 7.9, 10.9, 5-H$_b$), 1.64 (3H, s, CH_3), 1.40 (3H, s, CH_3); δ_C ($CDCl_3$) 138.6, 138.3 (Bn), 134.5 (C-1′), 128.3−127.4 (Bn), 118.2 (C-2′), 112.9 ($C(CH_3)_2$), 104.7 (C-1), 84.7, 81.1, 81.0 (C-2, C-3, C-4), 73.3 (C-5), 69.4, 67.0 (Bn), 26.8, 26.6 (CH_3).

1,2-Di-*O*-acetyl-3,5-di-*O*-benzyl-3-*C*-vinyl-α,*β*-D-ribofuranose (3a). A solution of furanose **3** (4.40 g, 11.1 mmol) in 80% aqueous acetic acid (50 cm^3) was stirred at 90 °C for 8 h. The solvents were removed, and the residue was coevaporated with 99% ethanol (3 × 25 cm^3), toluene (3 × 25 cm^3) and anhydrous pyridine (2 × 25 cm^3), and redissolved in anhydrous pyridine (20 cm^3). Acetic anhydride (17 cm^3) was added, and the solution was stirred at room temperature for 48 h. The reaction was quenched with ice-cold water (100 cm^3) and extracted with dichloromethane (2 × 100 cm^3). The combined extract was washed with saturated aqueous sodium hydrogencarbonate (3 × 100 cm^3) and dried (Na_2SO_4). The solvent was evaporated, and the residue was purified by silica gel column chromatography using petroleum ether/ethyl acetate (4:1, v/v) as eluent to give furanose **3a** as an oil (4.27 g, 87%, α:*β* ≈ 1:1). δ_C ($CDCl_3$) 169.9, 169.8 (C═O), 139.0, 138.6, 138.0, 137.8 (Bn), 133.3, 132.4 (C-1′), 128.4−126.8 (Bn), 119.6, 119.5 (C-2′), 99.5, 94.0 (C-1), 85.4, 85.0, 84.3, 83.6, 77.7, 73.6, 73.5, 73.3, 70.0, 69.2, 67.5, 67.2 (C-2, C-3, C-4, C-5, Bn), 21.0, 20.9, 20.6, 20.4 (CH_3).

1-(2-*O*-Acetyl-3,5-di-*O*-benzyl-3-*C*-vinyl-*β*-D-ribofuranosyl)thymine (3b). To a stirred solution of compound **3a** (4.24 g, 9.6 mmol) and thymine (2.43 g, 19.3 mmol) in anhydrous acetonitrile (100 cm^3) was added *N*,*O*-bis(trimethylsilyl)acetamide (11.9 cm^3, 48.1 mmol). The reaction mixture was stirred at reflux for 30 min. After cooling to 0 °C, trimethylsilyl triflate (3.2 cm^3, 16.4 mmol) was added dropwise, and the solution was stirred for 24 h at room temperature. The reaction was quenched with cold saturated aqueous sodium hydrogencarbonate (100 cm^3), and the resulting mixture was extracted with dichloromethane (3 × 50 cm^3). The combined extract was washed with saturated aqueous sodium hydrogencarbonate (2 × 50 cm^3) and brine (2 × 50 cm^3) and dried (Na_2SO_4). The extract was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give nucleoside **3b** as a white foam (4.03 g, 83%): δ_H ($CDCl_3$) 8.78 (1H, br s, NH), 7.75 (1H, s, 6-H), 7.38−7.26 (10 H, m, Bn), 6.49 (1H, d, *J* 8.1, 1′-H), 5.99−5.88 (2H, m, 2′-H and 1″-H), 5.54−5.48 (2H, m, 2″-H$_a$, 2″-H$_b$), 4.91−4.50 (4H, m, Bn), 4.34 (1H, s, 4′-H), 3.80 (1H, m, 5′-H$_a$), 3.54 (1H, m, 5′-H$_b$), 2.11 (3H, s, $COCH_3$), 1.48 (3H, s, CH_3); δ_C ($CDCl_3$) 170.1 (C═O), 163.8 (C-4), 151.0 (C-2), 138.9, 136.9 (Bn), 136.1 (C-6), 132.0 (C-1″), 128.7, 128.5, 128.2, 127.8, 127.7, 127.5, 127.5, 127.1 (Bn), 120.7 (C-2″), 111.3 (C-5), 85.4 (C-1′), 85.2 (C-3′), 84.3 (C-4′), 76.0 (C-2′), 73.7 (C-5′), 69.3, 67.6 (Bn), 20.6 ($COCH_3$), 11.7 (CH_3). Anal. Found: C, 66.3; H, 6.0; N, 5.1. Calcd for $C_{28}H_{30}N_2O_7$: C, 66.4; H, 6.0; N, 5.5.

1-(3,5-Di-*O*-benzyl-3-*C*-vinyl-*β*-D-ribofuranosyl)thymine (4). To a stirred solution of nucleoside **3b** (3.90 g, 7.7 mmol) in anhydrous methanol (40 cm^3) was added sodium methoxide (0.83 g, 15.4 mmol). The mixture was stirred at room temperature for 42 h and then neutralized with dilute aqueous hydrochloric acid. The mixture was extracted with dichloromethane (2 × 150 cm^3), and the combined extract was washed with saturated aqueous sodium hydrogencarbonate (3 × 100 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure to give nucleoside **4** as a white foam (3.48 g, 97%): δ_H ($CDCl_3$) 8.89 (1H, br s, NH), 7.60 (1H, d, *J* 0.9, 6-H), 7.36−7.26 (10H, m, Bn), 6.23 (1H, d, *J* 7.8, 1′-H), 5.98 (1H, dd, *J* 11.2, 17.7, 1″-H), 5.66 (1H, d, *J* 17.7, 2″-H$_a$), 5.55 (1H, d, *J* 11.5, 2″-H$_b$), 4.75−4.37 (6H, m, 2′-H, 4′-H, Bn), 3.84 (1H, dd, *J* 2.7, 10.8, 5′-H$_a$), 3.58 (1H, d, *J* 11.2, 5′-H$_b$), 3.23 (1H, d, *J* 10.6, 2′-OH), 1.50 (3H, s, CH_3); δ_C ($CDCl_3$) 163.7 (C-4), 151 3 (C-2), 138.0, 136.9 (Bn), 136.0 (C-6), 131.2 (C-1″), 128.8, 128.6, 128.3, 127.8, 127.7, 127.3 (Bn), 120.7 (C-2″), 111.3 (C-5), 87.3 (C-1′), 84.6 (C-3′), 81.4 (C-4′), 78.0 (C-2′), 73.7 (C-5′), 70.0, 66.4 (Bn), 11.8 (CH_3). Anal. Found: C, 66.8; H, 6.2; N, 5.9. Calcd for $C_{26}H_{28}N_2O_6$: C, 67.2; H, 6.1; N, 6.0.

1-(3,5-Di-*O*-benzyl-2-*O*-methanesulfonyl-3-*C*-vinyl-*β*-D-ribofuranosyl)thymine (4a). Nucleoside **4** (2.57 g, 5.53 mmol) was dissolved in anhydrous pyridine (18 cm^3) and cooled to 0 °C. Methanesulfonyl chloride (1.28 cm^3, 16.6 mmol) was added dropwise, and the mixture was stirred at room temperature for 30 min. The reaction was quenched with water (5 cm^3), and the resulting mixture was extracted with dichloromethane (3 × 80 cm^3). The combined extract was washed with saturated aqueous sodium hydrogencarbonate (3 × 120 cm^3) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give nucleoside **4a** as a yellow foam (2.53 g, 84%): δ_H ($CDCl_3$) 8.92 (1H, br s, NH), 7.71 (1H, d, *J* 1.4, 6-H), 7.41−7.28 (10H, m, Bn), 6.57 (1H, d, *J* 7.8, 1′-H), 5.99−5.61 (4H, m, 2′-H, 1″-H and 2″-H$_a$, 2″-H$_b$), 4.86−4.50 (4H, m, Bn), 4.37 (1H, dd, *J* 1.5, 2.4, 4′-H), 8.82 (1H, dd, *J* 2.6, 11.0, 5′-H$_a$), 3.55 (1H, dd, *J* 1.2, 11.0, 5′-H$_b$), 3.02 (3H, s, CH_3), 1.47 (3H, d, *J* 1.1, CH_3); δ_C ($CDCl_3$) 163.7 (C-4), 151.5 (C-2), 138.7, 136.7 (Bn), 135.7 (C-6), 130.9 (C-1″), 128.8, 128.5, 128.4, 127.6, 127.0 (Bn), 121.8 (C-2″), 111.9 (C-5), 85.1 (C-1′), 84.5 (C-3′), 84.0 (C-4′), 80.7 (C-2′), 73.7 (C-5′), 69.2, 67.7 (Bn), 38.9 (CH_3), 11.8 (CH_3).

1-(3,5-Di-*O*-benzyl-3-*C*-vinyl-*β*-D-arabinofuranosyl)thymine (4b). A solution of nucleoside **4a** (2.53 g, 4.66 mmol) in a mixture of ethanol (50 cm^3), water (50 cm^3), and 1 M aqueous sodium hydroxide (15 cm^3) was stirred under reflux for 16 h. The mixture was neutralized using dilute aqueous hydrochloric acid, the solvent was evaporated under reduced pressure, and the residue was extracted with dichloromethane (3 × 120 cm^3). The combined extract was washed with saturated

aqueous sodium hydrogencarbonate (3 × 150 cm^3) and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1) as eluent to give **4b** as a white foam (1.61 g, 74%): δ_H ($CDCl_3$) 9.89 (1H, br s, NH), 7.50 (1H, d, *J* 1.1, 6-H), 7.41−7.26 (Bn), 6.28 (1H, d, *J* 2.8, 1′-H), 6.05 (1H, dd, *J* 11.1, 17.9, 1″-H), 5.58−5.50 (2H, m, 2″-H_a, 2″-H_b), 4.98 (1H, d, *J* 9.0, 2′-OH), 4.64−4.31 (6H, m, 2′-H, 4′-H, Bn), 3.73 (2H, m, 5′-H_a, 5′-H_b), 1.73 (1H, d, *J* 0.6, CH_3); δ_C ($CDCl_3$) 165.1 (C-4), 150.5 (C-2), 138.4, 138.0, 136.7 (C-6, Bn), 130.4 (C-1″), 128.8, 128.6, 128.5, 128.1, 128.0, 127.8 (Bn), 120.6 (C-2″), 108.1 (C-5), 88.6 (C-1′), 87.9 (C-3′), 87.2 (C-4′), 73.7 (C-2′), 71.8 (C-5′), 69.7, 66.3 (Bn), 12.3 (CH_3). Anal. Found: C, 66.8; H, 6.2; N, 5.9. Calcd for $C_{26}H_{28}N_2O_6$: C, 67.2; H, 6.1; N, 6.0.

1-(3,5-Di-*O*-benzyl-3-*C*-(hydroxymethyl)-β-D-arabinofuranosyl)-thymine (5). To a solution of nucleoside **4b** (2.00 g, 4.31 mmol) in a mixture of THF (15 cm^3) and water (15 cm^3) were added sodium periodate (2.76 g, 12.9 mmol) and a 2.5% solution of osmium tetraoxide in *tert*-butyl alcohol (w/w, 0.54 cm^3, 43 μmol). The reaction was stirred at room temperature for 18 h, quenched with water (50 cm^3), and the mixture was extracted with dichloromethane (2 × 100 cm^3). The combined extract was washed with saturated aqueous sodium hydrogencarbonate (3 × 75 cm^3), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was redissolved in a mixture of THF (15 cm^3) and water (15 cm^3), and sodium borohydride (488 mg, 12.9 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, water (50 cm^3) was added, and the mixture was extracted with dichloromethane (2 × 100 cm^3). The combined organic phase was washed with saturated aqueous sodium hydrogencarbonate (3 × 75 cm^3) and dried (Na_2SO_4). The solvent was removed and the residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give nucleoside **5** as a white foam (732 mg, 36%): δ_H ($CDCl_3$) 11.09 (1H, br s, NH), 7.41 (1H, d, *J* 1.0, 6-H), 7.38−7.26 (Bn), 6.16 (1H, d, *J* 2.6, 1′-H), 5.12 (1H, d, *J* 5.4, 2′-OH), 4.66−4.29 (6H, m, 2′-H, 4′-H, Bn), 4.02−3.96 (2H, m, 1″-H_a, 1″-H_b), 3.90 (1H, dd, *J* 7.2, 9.7, 5′-H_a), 3.79 (1H, dd, *J* 5.6, 9.7, 5′-H_b), 2.49 (1H, t, *J* 6.4, 1″-OH), 1.68 (3H, d, *J* 0.6, CH_3); δ_C ($CDCl_3$) 166.1 (C-4), 150.6 (C-2), 139.0, 137.9, 137.0 (C-6, Bn), 128.7, 128.6, 128.4, 128.3, 128.0 (Bn), 107.5 (C-5), 88.2 (C-1′), 88.1 (C-3′), 84.2 (C-4′), 73.7 (C-5′), 72.1 (C-2′), 69.3, 65.4 (Bn), 58.6 (C-1″), 12.3 (CH_3).

(1*R*,2*R*,4*R*,5*S*)-1-(Benzyloxy)-2-(benzyloxymethyl)-4-(thymin-1-yl)-3,6-dioxabicyclo[3.2.0]heptane (5a). A solution of compound **5** (2.26 g, 4.83 mmol) in anhydrous pyridine (20 cm^3) was stirred at −40 °C, and a solution of methanesulfonyl chloride (0.482 cm^3, 4.83 mmol) in anhydrous pyridine (10 cm^3) was added. The reaction mixture was stirred at room temperature for 17 h, water (50 cm^3) was added, and the mixture was extracted with dichloromethane (2 × 100 cm^3). The combined organic phase was washed with saturated aqueous sodium hydrogencarbonate (3 × 100 cm^3), dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give an intermediate which after evaporation of the solvents was dissolved in anhydrous DMF (15 cm^3). This solution was added dropwise to a suspension of 60% sodium hydride (461 mg, 11.5 mmol) in anhydrous DMF (15 cm^3) at 0 °C. The reaction was stirred at room temperature for 30 min and then quenched with water (60 cm^3). After neutralization with dilute aqueous hydrochloric acid, dichloromethane (150 cm^3) was added, the resulting mixture was washed with saturated aqueous sodium hydrogencarbonate (3 × 100 cm^3), and the separated organic phase was dried (Na_2SO_4). The solvents were evaporated, and the residue was purified by silica gel column chromatography using dichloromethane/methanol (99:1, v/v) as eluent to give nucleoside **5a** as a white foam (2.00 g, 93%): δ_H ($CDCl_3$) 9.13 (1H, br s, NH), 7.55 (1H, d, *J* 1.4, 6-H), 7.40−7.26 (Bn), 5.99 (1H, d, *J* 2.5, 1′-H), 5.30 (1H, d, *J* 2.7, 2′-H), 4.88−4.57 (6H, m, 1″-H_a, 1″-H_b, Bn), 4.22−4.19 (1H, m, 4′-H), 3.92 (1H, dd, *J* 6.2, 10.8, 5′-H_a), 3.82 (1H, dd, *J* 3.7, 10.8, 5′-H_b), 1.91 (3H, d, *J* 1.3, CH_3); δ_C ($CDCl_3$) 163.8 (C-4), 150.3 (C-2), 137.6 (C-6), 137.5, 137.0 (Bn), 128.7, 128.6, 128.2, 128.0, 127.8, 127.3 (Bn), 109.8 (C-5), 85.7 (C-3′), 84.1 (C-1′), 83.5 (C-4′), 79.7 (C-1″), 73.9 (C-2′), 73.6 (C-5′), 68.6, 67.8 (Bn), 12.4 (CH_3); FAB *m*/*z* 451 $[M + H]^+$, 473 $[M + Na]^+$. Anal. Found: C, 66.3; H, 5.9; N, 6.1. Calcd for $C_{25}H_{26}N_2O_6$: C, 66.7; H, 5.8; N, 6.2.

(1*R*,2*R*,4*R*,5*S*)-1-Hydroxy-2-(hydroxymethyl)-4-(thymin-1-yl)-3,6-dioxabicyclo[3.2.0]heptane (6). To a stirred solution of nucleoside **5a** (180 mg, 0.40 mmol) in ethanol (3 cm^3) was added 10% palladium hydroxide over carbon (90 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 using dichloromethane/methanol (96:4, v/v) as eluent to give nucleoside **6** as a white solid material (92 mg, 86%): δ_H (CD_3OD) 7.79 (1H, d, *J* 1.2, 6-H), 5.91 (1H, d, *J* 2.5, 1′-H), 4.96 (1H, d, *J* 2.5, 2′-H), 4.92 (1H, d, *J* 7.4, 1″-H_a), 4.58 (1H, dd, *J* 0.9, 7.4, 1″-H_b), 3.98 (1H, dd, *J* 7.3, 12.8, 5′-H_a), 3.87−3.82 (2H, m, 4′-H, 5′-H_b), 3.34 (2H, s, 3′-OH, 5′-OH), 1.87 (3H, d, *J* 1.3, CH_3); δ_C (CD_3OD) 166.5 (C-4), 152.1 (C-2), 140.1 (C-6), 110.1 (C-5), 91.2 (C-2′), 85.1 (C-1′), 84.0 (C-4′), 79.6 (C-3′), 78.6 (C-1″), 61.1 (C-5′), 12.3 (CH_3).

(1*R*,2*R*,4*R*,5*S*)-1-(2-Cyanoethoxy(diisopropylamino)phosphinoxy)-2-(4,4′-dimethoxytrityloxymethyl)-4-(thymin-1-yl)-3,6-dioxabicyclo-[3.2.0]heptane (6a). To a solution of diol **6** (250 mg, 0.925 mmol) in anhydrous pyridine (4 cm^3) was added 4,4′-dimethoxytrityl chloride (376 mg, 1.11 mmol), and the mixture was stirred at room temperature for 18 h. The reaction was quenched with methanol (1.5 cm^3), and the mixture was evaporated under reduced pressure. A solution of the residue in dichloromethane (30 cm^3) was washed with saturated aqueous sodium hydrogencarbonate (3 × 20 cm^3), dried (Na_2SO_4), and evaporated. The residue was purified by silica gel column chromatography using dichloromethane/methanol (98:2, v/v) as eluent to give an intermediate which was dissolved in anhydrous dichloromethane (7.0 cm^3). *N*,*N*-Diisopropylethylamine (0.64 cm^3, 3.70 mmol) followed by 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.41 cm^3, 1.85 mmol) were added, and the mixture was stirred at room temperature for 25 h. The reaction was quenched with methanol (3 cm^3), and the mixture was dissolved in ethyl acetate (70 cm^3), washed with saturated aqueous sodium hydrogencarbonate (3 × 50 cm^3) and brine (3 × 50 cm^3), dried (Na_2SO_4), and was evaporated under reduced pressure. The residue was purified by silica gel column chromatography using petroleum ether/dichloromethane/ethyl acetate/triethylamine (100:45:45:10, v/v/v/v) as eluent. The residue obtained was dissolved in toluene (2 cm^3) and precipitated under stirring from petroleum ether at −50 °C. After evaporation of the solvents, the residue was coevaporated with anhydrous acetonitrile (4 × 5 cm^3) to give **6a** as a white foam (436 mg, 61%): ^{31}P NMR ($CDCl_3$) 146.6.

Oligonucleotide Synthesis and Analysis. Oligonucleotide analogues were synthesized on a 0.2 μmol scale, using unmodified 3′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5′-*O*-4,4′-dimethoxytrityl 2′-deoxynucleosides and amidite **6a** (stepwise coupling yield ∼95%, 12 min coupling), as described earlier.[3] Matrix-assisted laser desorption mass spectra were recorded to verify the molecular weight and monomer composition of all modified ONs. Capillary gel electrophoresis was performed to verify the purity (>90%) of all modified ONs. MALDI-MS $[M - H]^-$: 4225.3 (5′-T_7**X**T_6 calcd 4224.8); 4252.6 (5′-T_6**X**$_2T_6$ calcd 4252.8); 4251.0 (5′-T_6**X**T**X**T_5 calcd 4252.8); 4309.1 (5′-T_5**X**$_4T_5$ calcd 4308.8); 4308.7 (5′-T_3(**X**T)$_4T_3$ calcd 4308.8); 4560.6 (5′-**X**$_{13}$T calcd 4560.9); 2838.2 (5′-G**X**GA**X**A**X**GC calcd 2837.9).

Duplex Meltings and 3′-Exonucleolytic Stability Studies. These experiments were performed as previously described.[9]

Molecular Modeling. NMR Spectroscopy. 1D ^1H NMR spectra (CD_3OD) were recorded at 500 MHz in the temperature range of −50 to +50 °C in steps of 20 °C for both nucleotides **1** and **6**. **Model Building and Refinement Protocols.** The initial models of **1** and **6** were created with the Biopolymer module of INSIGHTII (version 95.0, Biosym/MSI, San Diego, CA). Potentials were assigned by the AMBER force field and partial charges were calculated in INSIGHTII. Molecular modeling was done using DISCOVER (version 95.0, Biosym/MSI, San Diego, CA). The refinement methods employed were restrained energy minimization (rEM) and restrained molecular dynamics (rMD). The rEM protocol consisted of 500 steps of steepest descent followed by conjugate gradient until a maximum rms derivative of 0.001 Å. The rMD procedure consisted of an initial energy minimization

followed by 28 ps of rMD. Dynamics were performed at 600 K for 4 ps, followed by cooling to 200 K in 50 K steps of 3 ps each. Finally, the structure was energy minimized to a maximum rms derivative of 0.01 Å. A distance dependent dielectric constant, $\epsilon = 4r$, was employed throughout the calculations. Experimentally derived angle restraints were incorporated into the refinement procedures as pseudo energy terms

$$E_{\text{exp}} = \begin{cases} k_1(\theta - \theta_1)^2 & \text{when } \theta_1 > \theta \\ 0 & \text{when } \theta_2 \geq \theta \geq \theta_1 \\ k_2(\theta - \theta_2)^2 & \text{when } \theta_2 > \theta \end{cases}$$

where k_1 and k_2 are the force constants and θ_1 and θ_2 are the lower and upper angle bounds as determined by the experimental data. Upper and lower force constants of 50 kcal/(mol Å^2) were employed. **Molecular Modeling of Nucleoside 6**. The $J_{1'2'}$ coupling constant was determined to 2.5 Hz. This $^3J_{\text{HH}}$ coupling constant was transferred to a dihedral angle (H1′−C1′−C2′−H2′) by the Karplus equation $^3J_{\text{HH}} = 10.2 \cos^2 \theta - 0.8 \cos \theta$.[15] This quadratic equation yields two solutions, and both angles were evaluated. One of them resulted in a highly strained conformation which consequently was discarded. The dihedral angle selected was incorporated into the refinement procedure as a flat well potential with a flat well width of 4.0° to reflect the approximate nature of the Karplus equation. To sample conformational space adequately, 24 starting structures with furanose ring conformations of $P = 15°n$, $n = 0$, ..., 23 were constructed. When subjected to rEM, all starting structures converged to just one family of structures with $P = 94° \pm 1°$ (O4′-endo conformation). **Molecular Modeling of Nucleoside 1.** The $J_{1'2'}$ coupling constant was determined to 4.3 Hz. Furthermore, the four $^3J_{\text{HH}}$ coupling constants between the H1″a, H1″b, H2″a, and H2″b protons in the other ring could also be determined by spectral simulation of the resulting ABMX system. Thus, a total of five dihedral angle restraints could be included in the structure refinement via the Karplus equation stated above. All restraints were represented by a flat well potential. For the furanose dihedral angle, a well width of 4.0° was employed while a broader potential well of 30° was chosen for the dihedral angle restraints in the other ring. A slightly different *modus operandi* was chosen for this nucleoside. Twenty-four structures corresponding to furanose ring conformations of P = 15°n, $n = 0$, ..., 23 were constructed, and scrutiny of these structures revealed that the experimental coupling constants could only be reproduced with a ring conformation near P = 135°. Consequently, this structure was chosen as starting structure for further refinement consisting of rMD as described above. Twenty final structures were generated which all converged to one family of structures with $P = 129° \pm 4°$ (C1′-exo conformation).

(15) Davies, D. B. *Prog. NMR Spectrosc.* **1978**, *12*, 135.

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Supporting Information Available: ^{13}C NMR spectra for compounds **2a**, **3**, **3a**, **3b**, **4**, **4a**, **4b**, **5**, **5a**, and **6** and a figure showing the preferred conformations for nucleosides **1** and **6** as found by molecular modeling (11 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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