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Synthesis and Pairing Properties of Oligodeoxynucleotides Containing Bicyclo-RNA and Bicyclo-ANA Modifications

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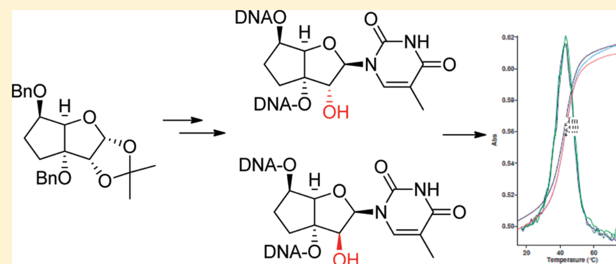
Supporting Information

ABSTRACT: The synthesis of the ribo(bc-rT)- and arabino(bc-araT)-version of bicyclothymidine (bc-dT) has been achieved. A conformational analysis by X-ray and/or ^{1}H NMR spectroscopy on the corresponding 3′,5′-benzyl-protected nucleosides featured a rigid C(2′)-endo conformation for the furanose ring, irrespective of the configuration of the OH group at C(2′). The conformation of the carbocyclic ring in these nucleosides was found to be less defined and thus more flexible. Both nucleosides were converted into the corresponding phosphoramidites and incorporated into oligodeoxynucleotides by standard DNA chemistry. T_m-data of duplexes with cDNA and RNA revealed that a bc-rT unit strongly destabilized duplexes with cDNA and RNA by 6−8 °C/mod, while bc-araT was almost T_m neutral. A rationale based on a previous structure of a bc-DNA mini duplex suggests that the strong destabilization caused by a bc-rT unit arises from unfavorable steric interactions of the equatorial 2′-OH group with the sugar residue of the 3′-neighboring nucleotide unit.



INTRODUCTION

The search for oligonucleotide-based therapeutics is an active and innovative field that has greatly evolved over the recent three decades.[1] In the early 1990s, the antisense approach was at the center of attention, in which a mRNA is targeted with an oligonucleotide to repress translation by either a steric block mechanism or an RNaseH-mediated ablation of the mRNA. More recent years have focused on the exploitation of further oligonucleotide-mediated RNA-silencing mechanisms such as RNA interference induced by small RNA duplexes (siRNAs)[2−5] or by targeting noncoding RNAs that are important in translational regulation, such as micro-RNAs, with single-stranded oligonucleotides (antimirs, antagomirs).[6−9]

Depending on the biological mechanism of action, it is believed that chemically modified oligonucleotides can outperform natural oligonucleotides because of increased biostability and increased target-RNA affinity via Watson−Crick pairing. In this context, numerous oligonucleotide analogues have been evaluated in the past, the biologically most successful ones being those where the ribonucleoside structure has been 2′-O-modified, such as 2′-MOE-RNA,[10] 2′F-RNA,[11] or 2′F-ANA,[12,13] those with modification in both the internucleosidic linkage and the nucleoside structure, such as morpholino-NAs,[14] or PNA,[15] or those with conformationally constrained nucleosides such as the LNA[16,17] or the HNA family.[18,19]

Our contribution to the field of conformationally constrained oligonucleotide analogues[20] in the past has been the development of the bicyclo(bc) and tricyclo(tc)-DNA molecular platforms.[21] In particular, tricyclo(tc)-DNA has shown very promising antisense results either as steric block inhibitor of HIV type 1 tat-dependent trans-activation,[22] as a splice modulator in cellular assays,[23] or as a dowregulator for scavenger receptor B1 mRNA in vivo in a gapmer format.[24] In order to address yet unsolved problems in oligonucleotide-based therapeutics, such as improving cellular uptake, we recently set out to extend the bc-DNA platform primarily by substituting position C-6′ with varying functional elements.[25,26] As part of this endeavor, we became interested in the ribo- (bc-RNA) and arabino- (bc-ANA) versions of bc-DNA, the rationale being to identify the influence of the 2′-OH group on nucleoside conformation and on nucleic acid affinity (Figure 1). While there exists a previous synthesis of an unprotected bc-rT nucleoside,[27,28] there is no data available on the synthesis of the bc-araT nucleosides or on oligonucleotides containing both nucleosides. Here we report on the synthesis of bicyclo ribothymidine (bc-rT) and bicyclo arabinothymidine (bc-araT), their structural and conformational preferences as determined by X-ray and/or ^{1}H NMR, their incorporation into

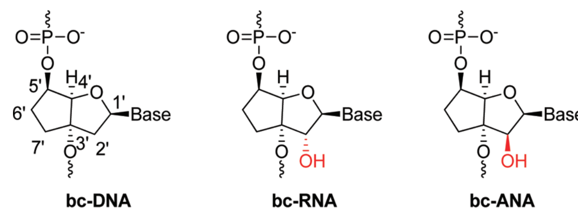


Figure 1. Chemical structures of the ribo- and arabino-version of bc-DNA.

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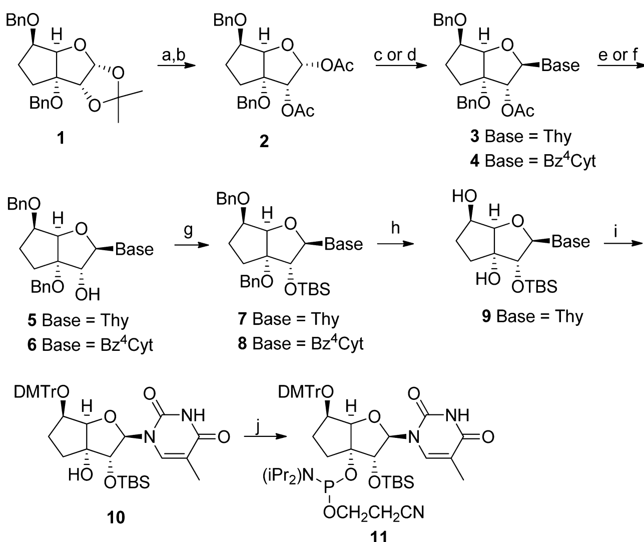
oligodeoxynucleotides, and finally on their pairing properties with cDNA and RNA.

■ RESULTS AND DISCUSSION

Synthesis of Nucleoside Building Blocks. The synthesis of the bicycloribo- and arabinonucleosides started with the sugar derivative **1**, which was prepared as reported previously (Scheme 1).[29] Acetonide **1**was hydrolyzed in boiling acetic acid followed by acetylation to give **2** that was subsequently converted into nucleosides **3** and **4** in excellent yield with in situ persilylated thymine or N-4-benzoylated cytosine and TMSOTf or $SnCl_4$ as Lewis acid promoter (Vorbrüggen conditions).[30,31] As expected, only the β-anomers could be isolated. The β-configuration at C(1′) in both nucleosides **3** and **4** was confirmed by ^{1}H NMR difference NOE experiments (see the Experimental Section). The 2′-OAc groups in **3** and **4** were cleaved with NaOMe in the case of **3** and with 0.2 M NaOH in a mixture of $THF/MeOH/H_2O$ in the case of the more delicate **4** to yield nucleosides **5** and **6** that were ready for the installation of a 2′-O-protecting group suitable for oligonucleotide synthesis. In preliminary experiments, we protected the 2′-OH with the triisopropylsilyloxy methyl (TOM) group.[32] We later found that this group was not stable during hydrogenolysis of the benzyl groups. We therefore switched to the alternative TBS group that was introduced using standard silylation conditions leading to **7** and **8** in excellent yields. Deprotection of the benzyl groups in **7** and **8**, however, proved to be rather tricky. Under standard conditions of hydrogenolysis (H_2, Pd/C) the reproducibility was limited, and partial or even complete decomposition was observed. Hydrogenation of the bases was another side reaction. After screening various palladium catalysts and different hydrogen sources and solvents, we found that H_2 and 1,3-cyclohexadiene as an additive with $Pd(OH)_2/C$ as catalyst in EtOAc worked best and gave the 2′-protected bc-rT nucleoside **9** reproducibly in yields >90%. Unfortunately, these and related conditions were unsuccessful for the deprotection of N^4-benzoyl bicycloribocytidine **8** without concomitant reduction of the base or loss of the benzoyl protecting group.

Scheme 1^a

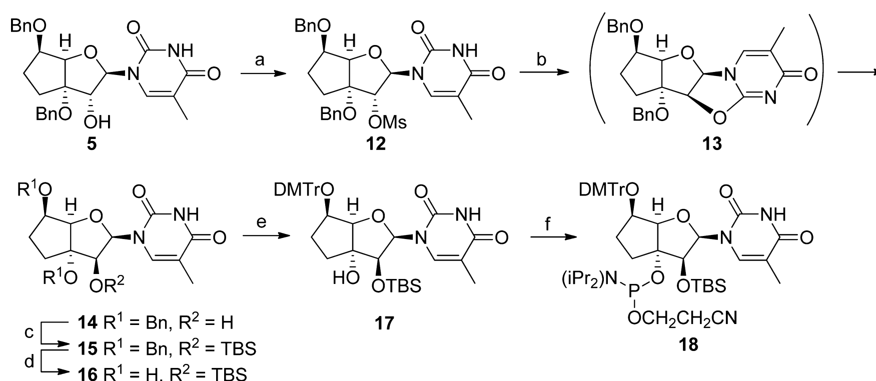


^{a}Reagents and conditions: (a) 80% aq AcOH, 90 °C, 16 h; (b) Ac_2O, pyridine, rt, 16 h, 74%; (c) thymine (2 equiv), BSA (5.3 equiv), TMS-OTf (2 equiv), MeCN, 50 °C, 16 h, 73%; (d) N^{4}Bz-Cytosine (2 equiv), BSA (5 equiv), $SnCl_4$ (3 equiv), MeCN, rt, 12 h, 98%; (e) NaOMe (2 equiv), MeOH, rt, 4 h, 91%; (f) 0.2 M NaOH/THF/MeOH, H_2O, 0 °C, 45 min, 48%; (g) TBS-Cl (1.3 equiv), imidazole (1.5 equiv), DMF, 24 h, **7** (66%), **8** (71%); (h) 1,3-cyclohexadiene (10 equiv), H_2, $Pd(OH)_2/C$, EtOAc, 6 h, 97%; (i) DMTr-Cl (6 equiv), pyridine, rt, 16 h, 90%; (j) $(iPr)_2NP(Cl)OCH_2CH_2CN$ (4 equiv), $EtN(iPr_2)_2$ (6 equiv), MeCN, rt, 4 h, 76%.

At this point, we decided not to optimize the conditions for benzyl deprotection of **8** but to continue with the synthesis of the phosphoramidite of bc-rT. Thus, selective tritylation of the 5′OH group with DMTr-Cl in **9** (→**10**), followed by standard phosphitylation with (2-cyanoethoxy)(diisopropylamino)-chlorophosphine, yielded the desired building block **11** in good yields.

The synthesis of the bicyclo arabinothymidine building block **18** was effected by inverting the configuration at C(2′) in nucleoside **5** using the "anhydro approach" (Scheme 2).[33] In initial experiments, **4** was treated with diphenyl carbonate to give the anhydro nucleoside **13** that was isolated and subsequently reacted with KOH to give the C(2′)-inverted arabino nucleoside **14**. However, the yields were low (20−30% for each step) and called for improvement of the procedure by converting the 2′-OH into a better leaving group. Therefore, **4**

Scheme 2^a



^{a}Reagents and conditions: (a) Ms-Cl (3 equiv), pyridine, rt, 1 h, 89%; (b) 1 M NaOH (4 equiv), EtOH, H_2O, reflux, 16 h, 85%; (c) TBS-OTf (1.5 equiv), imidazole (2.5 equiv), DMF, rt, 20 h, 71%; (d) 1,3-cyclohexadiene (10 equiv), H_2, $Pd(OH)_2/C$, EtOAc, 2 h, 58%; (e) DMTr-Cl (3 equiv), pyridine, rt, 5 h, 90%; (f) $(iPr)_2NP(Cl)OCH_2CH_2CN$ (3 equiv), $EtN(iPr_2)_2$ (5 equiv), MeCN, rt, 1 h, 97%.

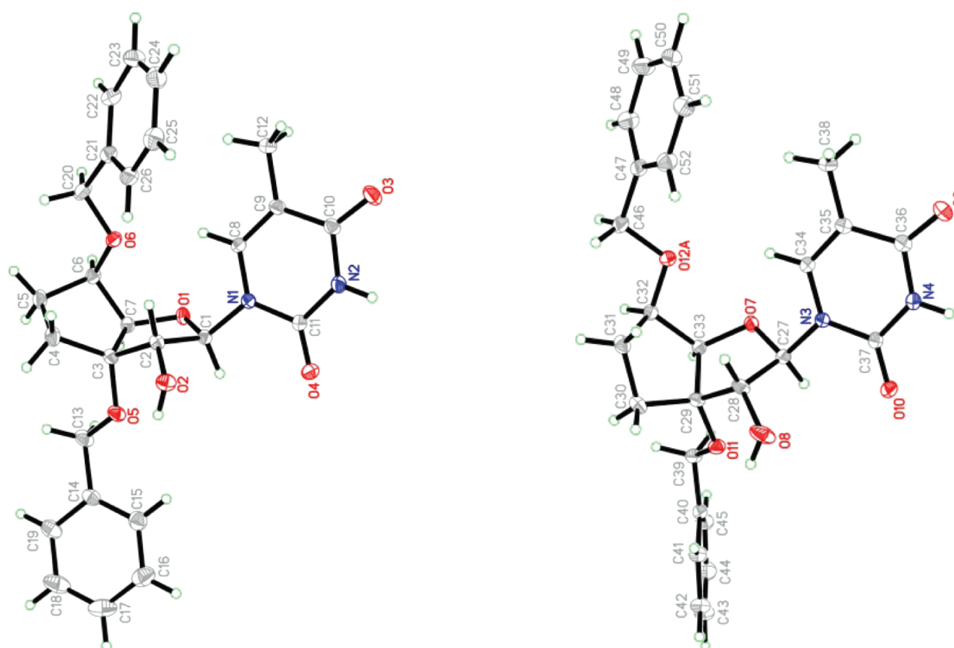


Figure 2. ORTEP (50% probability ellipsoids) representation of the two symmetry-independent molecules **5A** (left) and **5B** (right). H-atoms are given arbitrary displacement parameters for clarity.

was converted to the mesylate **12** that was subsequently treated with 1 M aq NaOH to give nucleoside **14** (via the anhydro intermediate **13**) in 85% yield. The arabino configuration in **14** was verified by ^{1}H NMR NOE experiments. As before, the 2′-OH group was TBS-protected to give **15**. Debenzylation as described before gave **16** in 58% that was subsequently tritylated (→ **17**) and phosphitylated (→ **18**) using standard conditions.

Conformational Properties of Nucleosides. *X-ray Structure of 5.* Crystals of benzyl-protected nucleoside **5** were subjected to X-ray analysis to map the conformational preferences of the bicyclic core structure. Two symmetry-independent molecules (**5A** and **B**) were found in the unit cell, which essentially differ only in the conformation of the carbocyclic ring. In molecule **5A** (Figure 2, left), the 5′-OBn group is in a pseudoaxial arrangement while in molecule **5B** (Figure 2, right) it is pseudoequatorially oriented. Thus, the torsion angle γ for **5A** is in a gauche (+*sc*) orientation as observed in A- and B-DNA, whereas for **5B** it is in an anticlinal (+*ac*) orientation, as observed for other bicyclonucleosides.[34,35] In the unit cell the 5′-OBn group of molecule **5A** almost perfectly stacks onto the nucleobase of molecule **5B**, suggesting that the orientation of O-5′ in **5A** may be biased by stacking interactions. Most interestingly, in both molecules **5A** and **5B**, the ribofuranose conformation is almost perfectly 2′-endo (South) giving rise to pseudorotation phase angles *P* of 165° for **5A** and 162° for **5B** (Table 1). Thus, the furanose conformation of bc-rT belongs to the same class as that of the 2′-deoxygenated derivative bc-dT, which shows a 1′-exo conformation with a *P* of 128°. This is somewhat unusual, as ribonucleosides typically prefer a 3′-endo (N) conformation. The orientation of the base (torsion angle χ) is, as expected, *anti.* Unfortunately, we were not able to get suitable crystals for X-ray analysis of **14** in order to compare the solid-state structures of the two diastereoisomers.

Table 1. Pseudorotational Phase Angles *P* and Selected Torsion Angles of bc-rT in Comparison to bc-dT[34]

nucleoside	furanose pucker	*P* (deg)	δ (deg)	γ (deg)	χ (deg)
5A	2′- endo (^{2}E)	165	150	75	−131
5B	2′- endo (^{2}E)	162	150	126	−145
bc-dT	1′- exo ($_1$E)	128	126	149	−113

Conformation of 5, 6 and 14 in Solution. Analysis of the vicinal coupling constants in the ^{1}H NMR spectra of the two ribo-configured nucleosides **5** and **6** and the arabino-configured **14** using the optimized Karplus relation for nucleosides[36] shed light on the conformation of the C(1′)−C(2′) and the C(5′)−C(6′) bonds (Table 2). A complete analysis of the sugar pucker of the bicyclo[3.3.0] skeleton was unfortunately not possible due to the coupling barrier at C(3′) and to substantial signal overlap of C(6′) and C(7′) in the carbocyclic subunit. However, an estimate for the population of the S-conformation in the ribo-configured nucleosides (Table 2) can be obtained from the following relationship

Table 2. Selected Vicinal Coupling Constants and Calculated Torsion Angles from ^{1}H NMR spectra (400 MHz) in CDCl$_3$

compd	coupling constants	^3J (Hz)	calcd dihedral angles (deg)	derived nucleoside torsion angles (deg)	% S conformation
5	$^3J_{H1'H2'}$	8.1	145	ν_1 = 35	80
	$^3J_{H5'H6'}$	5.1	35	γ = gauche	
	$^3J_{H5'H6''}$	4.9	37		
6	$^3J_{H1'H2'}$	6.6	137	ν_1 = 43	65
	$^3J_{H5'H6'}$	5.1	35	γ = gauche	
	$^3J_{H5'H6''}$	5.5	33		
14	$^3J_{H1'H2'}$	2.4	53	ν_1 = 37	
	$^3J_{H5'H6'}$	8.9	149	γ = anticlinal	

$$\%S = {}^3J_{H1'H2'} \times 100/10.1$$

assuming that the N- and S-conformation are the only populated conformations.[37]

The ν_1 values (O(4′)−C(1′)−C(2′)−C(3′)) calculated from the $^3J_{H1',H2'}$ data are in all three cases **5**, **6**, and **14** in agreement with the furanose unit adopting a C(2′)-endo conformation in solution ($CDCl_3$), indicating no large deviations from the solid state structure in this part of the molecule. The calculated population of the S conformation for the ribo-configured nucleosides **5** and **6** are 80% and 65%, respectively.

Within the carbocyclic ring we assume envelope conformations with two major conformers displaying the O(5′) substituent either in a pseudoaxial or pseudoequatorial position (Figure 3). In the former case, H-C(5′) is gauche to both H-C(6′). Hence, the coupling constants in such cases are expected to be between 3 and 4 Hz. If, however, O(5′) arranges pseudoequatorially, a trans-diaxial relationship with a large coupling constant (~10 Hz) between H-C(5′) and the β-H-C(6′) should be expected.

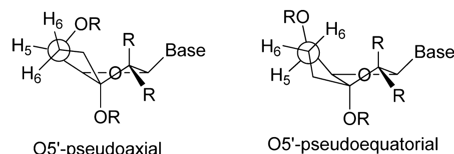


Figure 3. Two possible carbocyclic conformations with O(5′) in axial (left) and equatorial (right) arrangements.

The coupling constants $^3J_{H5',H6'}$ and $^3J_{H5',H6''}$, both around 5 Hz for **5** and **6**, are in agreement with a preference of the O(5′) for a pseudoaxial arrangement (γ = gauche). In the case of the arabinonucleoside **14** the situation is less clear since only one of the two $^3J_{H5'H6'}$ coupling constants could be resolved. Its magnitude around 9 Hz might be an indication for a higher preference of the pseudoequatorial (γ = anticlinal) arrangement of O(5′).

Oligonucleotide Synthesis. A series of oligonucleotide dodecamers containing single or double bc-rT or bc-araT substitutions (Table 3) were synthesized on a 1.3 μmol scale by standard phosphoramidite chemistry. For incorporation of the modified building blocks, the coupling step was extended to 12 min and the phosphoramidite concentration was increased to 0.2 M. Coupling efficiencies for building blocks **11** and **18** were generally lower (~90%) compared to that of unmodified nucleosides as determined by trityl assay. Crude oligonucleotides were deprotected and detached from the solid support by standard ammonia treatment followed by TBS removal with TBAF. All oligonucleotides were purified by HPLC, and their structural compositions were verified by ESI-MS (Supporting Information).

Table 3. T_m Data from UV-Melting Curves (260 nm) (Concentration (Total Single Strand) = 2 μM in 10 mM NaH_2PO_4, 150 mM NaCl, pH 7.0)

oligonucleotides	t =	DNA complement[a]	RNA complement[a]
5′-GGATGTTCTCGA-3′		47.5	49.5
5′-GGATGTTC**t**CGA-3′	bc-rT	39.3 (−8.1)	43.0 (−6.7)
	bc-araT	45.9 (−1.5)	48.4 (−1.2)
	bc-dT[b]	49.0 (+1.5)	49.0 (−0.5)
5′-GGATG**tt**CTCGA-3′	bc-rT	34.6 (−6.4)	41.0 (−4.3)
	bc-araT	42.6 (−2.4)	46.4 (−1.6)
	bc-dT[b]	48.7 (+0.6)	48.2 (−0.6)
5′-GGA**t**GTTC**t**CGA-3′	bc-rT	34.0 (−6.7)	39.3 (−5.1)
	bc-araT	42.4 (−2.5)	44.6 (−2.5)
	bc-dT[b]	47.9 (+0.2)	48.0 (−0.7)

[a] ΔT_m per modification relative to dT in parentheses. [b] Data taken from ref 38.

T_m Measurements. The UV melting curve analysis was performed at 260 nm with a cooling−heating−cooling cycle at a rate of 0.5 °C min^{-1} in standard saline buffer at pH 7.0. All curves within a cycle were superimposable, thus ruling out nonequilibrium association states. The measured T_m data are summarized in Table 3.

As seen here,there are considerable differences in nucleic acid affinities between the two 2′-epimeric bicyclonucleosides. Compared to a natural dT unit, the bc-araT modification shows only a modest destabilization by 1−2 °C per modification against both DNA and RNA as complements in a slightly sequence-dependent context. The bc-rT modification in contrast leads to a dramatic depression of T_m by 6−8 °C per modification against DNA and 4−7 °C against RNA. No 2′-OH group, as in the case of bc-dT, leads to subtle increases in T_m against DNA and modest decreases in T_m against RNA. As observed on other occasions, contiguous modifications lead to less T_m depression as noncontiguous modifications.

Some aspects of the presented results deserve further reflection. An important one concerns the conformation of the nucleosides as revealed by X-ray and/or NMR. Both the solid- and liquid-state structures of the two bc-RNA (**5**, **6**)and the one bc-ANA (**14**) nucleosides show a 2′-endo furanose conformation which is very similar to the 1′-exo conformation of bc-dT. These results confirm previous findings on the debenzylated derivative of **5**, where based on NMR analysis also a rigid 2′-endo conformation had been proposed.[27] Taken together, this strongly suggests that the specific structural features of the bicyclo[3.3.0] skeleton drives the furanose unit into a rigid south (S) type conformation, irrespective of the presence or the configuration of the OH group at C(2′). It thus overrules classical stereoelectronic factors (gauche effect) that are believed to be responsible for driving ribonucleosides preferentially into the 3′-endo conformation.[39] The fact that ribonucleosides prefer the 3′-endo conformation while that for arabinonucleosides is a more evenly distributed between S- and N-type is well documented on many examples.[40,41] While there seems to be a rigid furanose ring conformation, that of the carbocyclic ring in general seems to be structurally less defined in the bicyclonucleosides.

Perhaps the most striking result of this work is the strong destabilizing effect on duplex formation of a bc-RNA residue which is essentially absent in the case of a bc-ANA residue. This is in contrast to the natural system where RNA forms more stable duplexes with complementary RNA and DNA as compared to ANA, a fact that has been explained with steric obstruction of base-stacking caused by the β-OH group.[42] A possible structural rationale for this behavior may lie in the specific backbone conformation induced by the bicyclo scaffold. The only available high-resolution structure of bc-DNA is that of a bc-C dimer which was shown to form a parallel stranded C−C+ minihelix.[35] Surmising that the backbone structure would be similar in a classical antiparallel Watson−Crick duplex, modeling of a 2′-OH group into this structure in the ribo-configuration (Figure 4, left) clearly shows conflicting steric interactions between this hydroxyl group and either O(4′), O(5′), or C(6) of the neighboring 3′-nucleotide. We

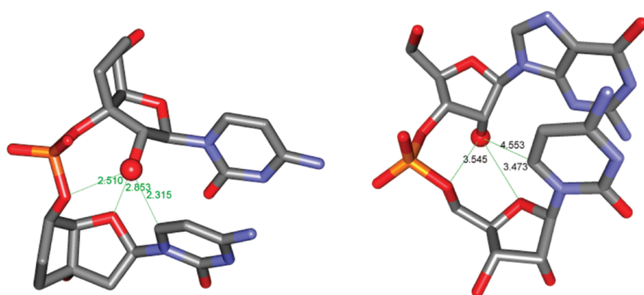


Figure 4. (Left) Structure of a bc-dinucleotide unit into which a 2′-OH group was modeled. The structure is based on an X-ray structure of the bc-C_2 dimer.[35] (Right) Exerpt of the X-ray structure of a standard RNA duplex (PDB ID: 3R1C).

therefore postulate that internucleotide steric repulsion of this equatorial hydroxy group is responsible for the strong destabilization. Such interactions are expected to be absent in the case of bc-DNA or bc-ANA residues which is well reflected in the corresponding T_m data. Interestingly, from a X-ray structure of a standard RNA-duplex in A-conformation (Figure 4, right, PDB ID: 3R1C) it appears that the axial 2′-OH group shows a distance of ~3.4 Å to O(4′) of the adjacent 3′-nucleotide. A hypothetic flipping of the 5′-ribonucleoside into a 2′-endo conformation would lead to steric conflicts between these two centers also in this case, suggesting that an equatorial 2′-OH substituent would negatively influence the pairing conformation also in a pure RNA backbone.

By comparing the pairing properties of ANA/RNA with DNA/RNA duplexes in a mixed sequence context it becomes evident that ANA/RNA duplexes are typically weaker by ca. 2 °C/mod.[42] These data compare well with the differential stability of bc-ANA/RNA and bc-DNA/RNA duplexes where we also find bc-ANA units to decrease duplex stability in the same range, suggesting that the influence of the β-2′-OH group on hybrid duplex stability (particularly on base-stacking) are similar in both backbone systems.

■ CONCLUSIONS

We present here the synthesis of two novel members of the bicyclo[3.3.0] family, namely the ribo and arabino versions of bicyclothymidine. Incorporation into oligodeoxynucleotides followed by thermal melting experiments revealed a strong destabilizing effect of a bicyclo-RNA unit and an almost T_m-neutral effect of a bicyclo-ANA unit in base-pairing with DNA and RNA complements. The destabilizing effect of a bc-RNA unit was rationalized by its preferred 2′-endo furanose conformation which places the 2′-OH group into an equatorial position, causing intrastrand steric conflicts with the 3′-neighboring nucleoside unit. Together with the structural analysis of the monomeric nucleosides this highlights the propensity of the bicyclo[3.3.0] scaffold to maintain the south conformation of the furanose unit irrespective of the substitution pattern at C(2′).

An interesting extension of this work is to study the effect of bicyclo-F-RNA or -F-ANA nucleosides in duplex formation. Recent work on F-RNA[43] and F-ANA[13,44] as well as on F-HNA[45] has shown a dramatic stabilization exerted by the fluorine substituent, most likely due to electrostatic effects on base-pairing. Work into this direction is currently in progress.

■ EXPERIMENTAL SECTION

General Methods. All reactions were performed under an atmosphere of argon in oven-dried glassware. Anhydrous solvents for reactions were obtained by filtration through activated alumina or by storage over molecular sieves (4 Å). Column chromatography (CC) was performed on silica gel with an average particle size of 40 μm. All solvents for CC were of technical grade and distilled prior to use. Thin-layer chromatography (TLC) was performed on silica gel plates. Visualization was achieved either under UV light or by staining in dip solutions (vanilline (15 g), ethanol (250 mL), concd H_2SO_4 (2.5 mL), *p*-anisaldehyde (10 mL), concentrated H_2SO_4 (10 mL), concentrated acetic acid (2 mL), or ethanol (180 mL)) followed by heating with a heat gun. NMR spectra were recorded at 300 or 400 MHz (^1H), at 75 MHz (^{13}C), and at 162 MHz (^{31}P). Chemical shifts (δ) are reported relative to the undeuterated residual solvent peak [$CHCl_3$: 7.27 ppm (^1H) and 77.0 ppm (^{13}C); CHD_2OD: 3.35 ppm (^1H) and 49.3 ppm (^{13}C)]. Signal assignments are based on DEPT or APT experiments, and on $^1H,^1H$- and $^1H,^{13}C$-correlation experiments (COSY/HMSC). ^1H NMR difference-NOE experiments were recorded at 400 MHz. Chemical shifts for ^{31}P NMR are reported relative to 85% H_3PO_4 as external standard. Electrospray ionization in the positive mode (ESI^+) was used for high-resolution mass detection.

(2*RS*,3*R*,3a*R*,6*R*,6a*R*)-3a,6-Bis(benzyloxy)-2-methoxyhexahydro-2*H*-cyclopenta[*b*]furan-3-yl Acetate (2). Compound **1** (1.0 g, 2.51 mmol) was dissolved in 80% aqueous AcOH (40 mL) and stirred at 90 °C for 16 h. AcOH was removed by coevaporation with EtOH (3 × 10 mL), toluene (3 × 10 mL), and anhydrous pyridine (1 × 10 mL). The residue was dissolved in anhydrous pyridine (10 mL), and Ac_2O (10 mL) was added dropwise. After the mixture was stirred at rt for 16 h, the reaction was quenched by the addition of water (20 mL) at 0 °C. The aqueous solution was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were washed with satd aq $NaHCO_3$ (2 × 50 mL), dried over $MgSO_4$, filtered, and evaporated. CC (hexane/EtOAc 2:1) yielded compound **2** (0.820 g, 74%) as a 3:1 mixture of α- and β-anomers in the form of a colorless oil. TLC (hexane/EtOAc 2:1): R_f 0.31 (β-anomer), 0.25 (α-anomer). ^1H NMR (300 MHz, $CDCl_3$), (data of β-anomer): δ 2.00–2.30 (m, 4H, 2H-4, 2H-5), 2.06, 2.15 (2s, 6H, $2CH_3$), 3.94–4.02 (m, 1H, H-6), 4.28 (d, *J* = 11.5 Hz, 1H, $1CH_2Ph$), 4.64 (d, *J* = 4.0 Hz, 1H, H-6), 4.68 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 5.50 (d, *J* = 11.5 Hz, 1H, 1 CH_2Ph), 5.51 (d, *J* = 11.8 Hz, 1H, 1 CH_2Ph), 5.28 (d, *J* = 0.75 Hz, 1H, H-3), 6.25 (s, 1H, H-2), 7.28–7.33 (m, 10H, Ph). ^1H NMR (300 MHz, $CDCl_3$); data of α-anomer: δ 1.85, 2.15 (2s, 6H, 2 CH3), 1.75–2.00 (m, 4H, 2H-4, 2H-5), 3.90–4.05 (m, 1H, H-6), 4.13 (dd, *J* = 7.1 Hz, *J* = 14.3 Hz, 1H, H-6), 4.52–4.70 (m, 4H, $2CH_2Ph$), 5.6 (d, *J* = 4.5 Hz, 1H, H-3), 6.52 (d, J = 4.5 Hz, 1H, H-2), 7.28–7.33 (m, 10H, Ph). ^{13}C NMR (75 MHz, $CDCl_3$, anomeric mixture): δ 21.1, 21.4, 21.7, 21.8, 28.9, 29.1, 30.6, 32.4, 68.0, 68.2, 72.6, 78.8, 79.1, 79.7, 79.8, 85.4, 88.0, 88.1, 90.1, 96.4, 101.7, 127.2, 127.6, 128.1, 128.3, 128.5, 128.5, 128.5, 129.0, 129.05, 129.11, 138.58, 138.62, 138.9, 139.5, 169.8, 170.0, 170.1. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{25}H_{28}O_7Na$ 463.1733, found 463.1728.

(3′*S*,5′*R*)-1-(2′-Acetoxy-3′,5′-*O*-dibenzyl-3′,5′-ethano-β-D-ribofuranosyl)thymidine (3). Compound **2** (0.80 g, 1.8 mmol) and dry thymine (0.45 g, 3.6 mmol) were suspended in anhydrous CH_3CN (35 mL). *N*,*O*-Bis(trimethylsilyl)acetamide (BSA) (1.9 g, 9.6 mmol) was added dropwise, and the solution was stirred at 80 °C for 1 h. The mixture was then cooled to 0 °C, and TMS-triflate (0.8 g, 3.6 mmol) was added. The clear solution was stirred at 50 °C for 16 h. The solution was then cooled to 0 °C and quenched by the addition of sat aq $NaHCO_3$ (30 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried with $MgSO_4$, filtered, and evaporated. CC (EtOAc/hexane 1:1) yielded nucleoside **3** (0.68 g, 73%, β anomer only) as a colorless foam. TLC (hexane/EtOAc 1:1): R_f 0.23. ^1H NMR (300 MHz, $CDCl_3$): δ 1.51 (d, J = 1.2 Hz, 3H, (CH_3)C-5, 2.07 (s, 3H, CH_3), 2.00–2.34 (m, 4H, 2H-6′, 2H-7′), 4.00–4.06 (m, 1H, H-5′), 4.57–4.73 (m, 5H, H-4′, $2CH_2Ph$), 5.15 (d, *J* = 8.1 Hz, 1H, H-2′), 6.45 (d, *J* = 8.1 Hz, 1H, H-1′), 7.28–7.39 (m, 10H, Ph), 7.54 (d, *J* = 1.3 Hz, 1H, H-6), 8.07 (s, 1H, NH). ^1H NMR-difference NOE (400 MHz, $CDCl_3$): 6.45 (H-1′) → 5.15 (H-2′, 2.1%), 4.63 (H-4′, 3.7%); 5.15 (H-2) → 7.54 (H-6, 12.3%), 6.45 (H-1′, 3.6%);

4.63 (H-4′) → 6.45 (H-1′, 2.8%), 4.06 (H-5′, 5.4%); 4.06 (H-5′) → 4.63 (H-4′, 15.6%). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.0, 20.7, 29.9, 30.2, 67.5, 71.5, 76.6, 78.1, 79.3, 86.7, 87.1, 88.8, 111.7, 126.8, 127.7, 128.1, 128.5, 128.7, 135.3, 137.6, 138.3, 150.4, 163.2, 170.5. HRMS (ESI): *m*/*z* calcd for $C_{28}H_{30}O_7N_2Na$ $(M + Na)^+$ 529.1951, found 529.1948.

(3′*S*,5′*R*)-*N*^{4}-Benzoyl-1-(2′-acetoxy-3′,5′-*O*-dibenzyl-3′,5′-ethano-*β*-D-ribofuranosyl)cytidine (4). Dry N^4-Bz-cytosine (0.19 g, 0.9 mmol) and acetylated sugar **2** (0.2 g, 0.45 mmol) were suspended in anhydrous CH_3CN (6 mL) and treated with BSA (0.45 g, 2.2 mmol). The suspension was stirred for 1 h at rt. Then, $SnCl_4$ (0.23 g, 0.9 mmol) was added dropwise, and the mixture was stirred for another 3 h at rt. Another 1 equiv of $SnCl_4$ (0.11 g, 0.45 mmol) was added and the mixture stirred overnight. The reaction was quenched with satd aq $NaHCO_3$ (10 mL) and the mixture extracted with CH_2Cl_2 (3 × 20 mL). The organic phases were washed with satd aq $NaHCO_3$ (3 × 15 mL) and brine (1 × 15 mL). The aqueous phase was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic phases were dried over $MgSO_4$, evaporated, and purified by CC (hexane/EtOAc 1:2) to give **4** (0.267 g, 98%) as a yellow foam. TLC (hexane/EtOAc 1:2): R_f 0.42. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04−2.25 (m, 7H, CH_3, 2H-6′, 2H-7′), 4.08 (m, 1H, H-5′), 4.58−4.70 (m, 5H, $2CH_2Ph$, H-4′), 5.17 (d, *J* = 7.3 Hz, 1H, H-2′), 6.63 (d, *J* = 7.3 Hz, 1H, H-1′), 7.28−7.70 (m, 14H, 13Ph, H-5), 7.98 (d, *J* = 7.4 Hz, 2H, 2Bz), 8.28 (d, *J* = 7.4 Hz, 1H, H-6), 8.62 (sb, 1H, NH). ^{1}H NMR-NOE (400 MHz, $CDCl_3$): 6.63 (H-1′) → 5.17 (H-2′, 2.6%), 4.71 (H-4′, 5.5%), 4.08 (H-5′, 0.2%); 4.08 (H-5′) → 4.70 (H-4′, 11.1%). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.7, 22.7, 30.5, 67.6, 71.7, 78.4, 81.0, 87.3, 89.0, 89.5, 126.9, 127.6, 128.2, 128.4, 128.5, 128.8, 129.0, 133.1, 137.3, 138.3, 170.4. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{34}H_{34}O_7N_3$ 596.2397, found 596.2393.

(3′*S*,5′*R*)-1-(2′-Hydroxy-3′,5′-*O*-dibenzyl-3′,5′-ethano-*β*-D-ribofuranosyl)thymidine (5). To a solution of nucleoside **3** (0.67 g, 1.3 mmol) in anhydrous MeOH (30 mL) was added NaOMe (0.14 g, 2.6 mmol). The clear solution was stirred at rt for 4 h. After being cooled to 0 °C, the solution was neutralized with aq HCl and then extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with satd aq $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated. CC (hexane/EtOAc 2:3) yielded compound **5** (0.560 g, 91%) as a white foam. TLC (EtOAc/hexane 3:2): R_f 0.18. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50 (s, 3H, CH_3), 2.04−2.13 (m, 4H, 2H6′, 2H7′), 3.07 (d, *J* = 10.3 Hz, 1H, 2′−OH), 3.98−4.10 (m, 2H, H-5′, H-2′), 4.56−4.67 (m, 5H, $2CH_2Ph$, H-4′), 6.15 (d, *J* = 8.1 Hz, 1H, H-1′), 7.28−7.45 (m, 10H, Ph), 7.49 (s, 1H, H-6), 8.20 (s, 1H, NH). ^{1}H NMR-NOE (400 MHz, $CDCl_3$): 6.15 (H-1′) → 4.64 (H-4′, 2.3%), 4.06 (H-2′, 1.3%); 4.65 (H-4′) → 4.04 (H-5′, 5.3%); 4.06 (H-2′) → 8.20 (N−H, 1.3%), 7.49 (C−H, 8.5%), 6.15 (H-1′, 1.7%). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.6, 26.8, 29.7, 65.8, 71.3, 77.7, 79.3, 83.2, 88.2, 88.6, 111.2, 127.1, 127.3, 127.8, 128.4, 128.4, 135.1, 137.0, 137.2, 150.5, 162.7. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{26}H_{28}O_6N_2Na$ 487.1845, found 487.1843.

(3′*S*,5′*R*)-*N*^{4}-Benzoyl-1-(2′-hydroxy-3′,5′-*O*-dibenzyl-3′,5′-ethano-*β*-D-ribofuranosyl)cytidine (6). Nucleoside **4** (0.26 g, 0.44 mmol) was dissolved in 0.2 M NaOH in THF/MeOH/H_2O 5:4:1 (25 mL) at 0 °C. After 45 min, the reaction was quenched by addition of NH_4Cl (0.347 g, 1.5 equiv relative to NaOH). The solution was stirred for another 10 min at rt before evaporation. The residue was adsorbed on silica gel (MeOH) and purified by CC (hexane/EtOAc 1:3) to give compound **6** (0.120 g, 52%) as a white foam. TLC (hexane/EtOAc 1:3): R_f 0.22. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.00−2.12 (m, 4H, 2H-6′, 2H-7′), 4.06 (m, 2H, H-2′, H-5′), 4.16 (sb, 1H, 2′−OH), 4.52−4.82 (m, 5H, $2CH_2Ph$, H-4′), 6.19 (d, *J* = 6.6 Hz, 1H, H-1′), 7.28−7.61 (m, 14H, 13Ph, 1H−C5), 7.89 (d, *J* = 7.4 Hz, 2H, H-Bz), 8.27 (d, *J* = 7.5 Hz, 1H, H-6), 8.68 (sb, 1H, NH). ^{1}H NMR-NOE (400 MHz, $CDCl_3$): 6.20 (H-1′) → 4.12 (H-2′, 3.3%); 4.78 (H-4′) → 6.23 (H-1′, 2.5%), 4.09 (H-5′, 9.9%). ^{13}C NMR (75 MHz, $CDCl_3$): δ 1.0, 14.1, 22.7, 28.7, 29.3, 29.5, 29.63, 29.67, 30.2, 31.4, 31.9, 67.2, 71.7, 78.2, 82.9, 86.5, 89.9, 93.0, 96.7, 113.6, 127.45, 127.52, 127.8, 128.1, 128.3, 128.5, 128.7, 129.1, 133.2, 137.4, 138.1, 144.9, 162.1. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{32}H_{32}O_6N_3$ 554.2291, found 554.2291.

(3′*S*,5′*R*)-1-(2′-*O*-*tert*-Butydimethylsilyl-3′,5′-*O*-dibenzyl-3′,5′-ethano-*β*-d-ribofuranosyl)thymidine (7). To a solution of **5** (200 mg, 0.43 mmol) in DMF (1 mL) were added imidazole (43 mg, 0.64 mmol) and TBS-Cl (84 mg, 0.56 mmol) at 0 °C. After being stirred at rt for 24 h, the mixture was diluted with $CHCl_3$ (25 mL) and washed with water (3 × 25 mL) and brine (25 mL). The combined organic phases were dried over $MgSO_4$ and evaporated, and the residue was purified by CC (hexane/EtOAc 2:3 + 1% Et_3N) to give **7** (0.166 g, 66%) as a yellow oil. TLC (hexane/EtOAc 2:3): R_f 0.66. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.08 (s, 3H, Si-CH_3), 0.09 (s, 3H, Si-CH_3), 0.85 (s, 9H, ^{t}Bu), 1.43 (s, 3H, CH_3(C-5)), 2.00−2.16 (m, 4H, 2H-6′, 2H-7′), 4.01−4.03 (m, 1H, H-5′), 4.13 (d, *J* = 8.1 Hz, 1H, H-2′), 4.53 (d, *J* = 5.3, 1H, H-4′), 4.55−4.97 (m, 4H, CH_2Ph), 6.38 (d, *J* = 8.3 Hz, 1H, H-1′), 7.28−7.40 (m, 10H, Ph), 7.52 (d, *J* = 1.3 Hz, 1H, H-6), 8.00 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.8, −4.0, 12.0, 14.5, 18.1, 21.3, 25.8, 25.9, 30.0, 30.1, 30.4, 60.6, 68.6, 71.6, 78.5, 81.2, 87.4, 88.7, 88.8, 116.8, 127.2, 127.7, 127.9, 128.3, 128.6, 128.9, 136.0, 138.1, 139.4, 150.6, 163.5. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{32}H_{43}O_6N_2Si$ 579.2890, found 579.2868.

(3′*S*,5′*R*)-*N*^{4}-Benzoyl-1-(2′-*O*-*tert*-butydimethylsilyl-3′,5′-*O*-dibenzyl-3′,5′-ethano-*β*-D-ribofuranosyl)cytidine (8). To a solution of **6** (90 mg, 0.16 mmol) in DMF (1 mL) were added imidazole (16 mg, 0.24 mmol) and TBS-Cl (31 mg, 0.21 mmol) at 0 °C. After being stirred for 24 h at rt, the mixture was diluted with $CHCl_3$ (25 mL) and washed with water (3 × 20 mL) and with brine (1 × 20 mL). The organic phase was dried over $MgSO_4$ and evaporated and the residue purified by CC (hexane/EtOAc 1:2 + 1% Et_3N) to give **8** (0.076 g, 71%) as a white solid. TLC (hexane/EtOAc 1:3): R_f 0.65. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.09 (s, 3H, Si-CH_3), 0.06 (s, 3H, Si-CH_3), 0.85 (s, 9H, ^{t}Bu), 1.52−2.22 (m, 4H, 2H-6′, 2H-7′), 4.10 (m, 2H, H-2′, H-5′), 4.50−4.95 (m, 5H, $2CH_2Ph$, H-4′), 6.60 (d, *J* = 7.9 Hz, 1H, H-1′), 7.28−7.60 (m, 14H, 13H-Ph, H-5), 7.92 (sb, 2H-Bz), 8.18 (d, *J* = 7.5 Hz, 1H, H-6), 8.70 (sb, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.0, −4.4, 1.0, 14.2, 17.8, 21.0, 25.6, 29.7, 30.6, 60.4, 68.3, 71.6, 78.6, 83.0, 87.8, 89.0, 90.1, 127.0, 127.4, 128.3, 128.4, 128.5, 128.9, 129.0, 133.3, 137.4, 139.1. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{38}H_{46}O_6N_3Si$ 668.3156, found 668.3157.

(3′*S*,5′*R*)-1-(3′,5′-Dihydroxy-2′-O-(*tert*-butyldimethylsilyl)-3′,5′-ethano-*β*-D-ribofuranosyl)thymidine (9). To a solution of **7** (0.18 g, 0.3 mmol) in EtOAc (4.5 mL) were added 20% $Pd(OH)_2$/C (0.1 g) and 1,3-cyclohexadiene (0.24 g, 0.3 mmol). The mixture was flushed with Ar for 15 min and then set under an atmosphere of H_2. After being stirred for 6 h at rt, the mixture was filtered through a pad of Celite and the filtrate evaporated. The residue was purified by CC (hexane/EtOAc 1:3) to yield **9** (0.12 g, 97%) as a white foam. TLC (hexane/EtOAc 1:3): R_f 0.26. ^{1}H NMR (300 MHz, MeOD): δ −0.11 (s, 3H, Si-CH_3), −0.17 (s, 3H, Si-CH_3), 0.92 (s, 9H, ^{t}Bu), 1.39−1.52 (m, 1H, 1H-7′), 1.70−1.85 (m, 1H, 1H-6′), 1.90 (s, 3H, (CH_3)C-5), 2.05−2.10 (m, 1H,1H-6′), 2.25−2.39 (m, 1H, 1H-7′), 3.80 (d, *J* = 5.1 Hz, 1H, H-4′), 3.97 (d, *J* = 3.6 Hz, 1H, H-2′), 4.05 (m, 1H, H-5′), 5.91 (d, *J* = 3.6 Hz, 1H, H-1′), 7.57 (s, 1H, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.9, −4.7, 12.7, 19.1, 26.7, 32.2, 33.7, 58.6, 73.5, 81.6, 87.4, 89.0, 89.6, 109.9, 141.3, 152.3, 166.7. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{31}O_6N_2Si$ 399.1951, found 399.1960.

(3′*S*,5′*R*)-1-[3′-Hydroxy-5′-O-[(4,4′-dimethoxytriphenyl)-methyl]-2′-O-(*tert*-butyldimethyllsilyl)-3′,5′-ethano-*β*-D-ribofuranosyl]thymidine (10). To a stirred solution of nucleoside **9** (0.29 g, 0.74 mmol) in pyridine (3 mL) was added DMTr-Cl (0.75 g, 2.2 mmol) at rt. After 3 h, another portion of DMTr-Cl (0.75 g, 2.2 mmol) was added and the mixture stirred for 16 h. The reaction was then quenched with satd aq $NaHCO_3$ (5 mL) and the mixture extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated, and the residue was purified by CC (hexane/EtOAc 2:1 + 1% Et_3N) to give **10** (0.47 g, 90%) as a yellow foam. TLC (hexane/EtOAc 2:1): R_f 0.30. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.02 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3), 0.87 (s, 9H, ^{t}Bu), 1.06- 2.23 (m, 7H, (CH_3)C-5, 2H-6′, 2H-7), 3.11 (s, 1H, 3′-OH), 3.78 (s, 6H, 2MeO), 3.94 - 3.96 (m, 2H, H-4′, H-2′), 4.00−4.10 (m, 1H, H-5′), 6.03 (d, *J* = 7.4 Hz, 1H, H-1′), 6.84 (dd, *J* = 9.0, 2.2 Hz, 4H, H-Ph), 7.28−7.49 (m, 10H, H-Ph, H-6), 8.12 (sb, 1H,

NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.7, −4.6, −4.5, −4.4, 12.0, 12.5, 14.3, 18.0, 22.9, 25.4, 29.5, 29.8, 34.7, 55.3, 71.5, 73.7, 79.8, 82.8, 87.5, 87.9, 88.2, 89.2, 94.3, 112.8, 113.4, 123.6, 127.3, 128.0, 128.6, 129.3, 130.5, 134.2, 136.5, 138.1, 139.7, 150.4, 158.3, 159.0, 163.9. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{39}H_{48}O_8N_2SiNa$ 723.3078, found 723.3087.

(3′S,5′R)-1-{3′-O-[(Cyanoethoxy)(diisopropylamino)-phosphino]-5′-O-[(4,4′-dimethoxytriphenyl)methyl]-2′-O-(tert-butyldimethyllsilyl)-3′,5′-ethano-β-D-ribofuranosyl)]thymidine (11). To a stirred solution of **10** (0.47 g, 0.67 mmol) in MeCN (3 mL) was added at rt $^iPr_2NEt$ (0.5 g, 0.4 mmol), followed by $^iPr_2NP(Cl)$-OCH_2CH_2CN (0.58 g, 0.26 mmol). After 4 h, the mixture was diluted with EtOAc (10 mL) and washed with sat aq $NaHCO_3$ (10 mL). The aqueous phase was extracted with EtOAc (3 × 10 mL) and the combined organic phase dried over $MgSO_4$, evaporated and the residue purified by CC (hexane/EtOAc 2:1 + 1% Et_3N) to obtain **11** (0.46 g, 76%) as a white foam. TLC (hexane/EtOAc 2:1): R_f 0.34. ^1H NMR (300 MHz, $CDCl_3$): δ 0.00, 0.01, 0.08, 0.13 (4s, 6H, Si-CH_3), 0.87, 0.88 (2s, 9H, ^tBu), 0.75−0.95 (m, 2H, 2H-6′), 1.20−1.35 (m, 12H, 4N−CH_3), 1.85−1.95 (m, 2H, 2H-7′),2.53−2.65 (m, 2H CH_2−CN), 3.40−3.98 (m, 10H, 2OMe, 2CH_2−O, 2 x CH-N), 4.10−4.21 (m, 2H, H-2′, H-5′), 4.34−4.37, 4.55−4.58 (2 m, 1H, H-4′), 6.22−6.26 (m, 1H, H-1′), 6.80−6.85 (m, 4H, Ph), 7.28−7.47 (m, 9H, Ph), 7.54 (s, 1H, H-6), 8.23 (bs, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ −4.7, −4.6, −3.8, 11.4, 14.4, 18.1, 18.2, 20.4, 20.6, 20.7, 24.4, 24.48, 24.51, 24.6, 24.7, 24.77, 24.82, 25.7, 25.8, 43.3, 43.4, 43.5, 43.6, 58.4, 72.8, 73.2, 87.6, 87.77, 87.84, 111.5, 111.6, 113.4, 117.9, 118.1, 127.46, 127.50, 128.1, 128.8, 130.6, 135.9, 136.0, 136.1, 136.2, 136.3, 136.4, 145.0, 145.1, 150.5, 150.6, 159.1, 163.5. ^{31}P NMR (162 MHz, $CDCl_3$): δ 141.2, 142.1. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{48}H_{66}O_9N_4$ PSi 901.4337, found 901.4352.

(3′S,5′R)-2,2′-Anhydro-1-(3′,5′-O-dibenzyl-3′,5′-ethano-β-D-arabinofuranosyl)thymidine (13). A solution of **5** (50 mg, 0.1 mmol) in DMF (2 mL) was treated with diphenyl carbonate (27 mg, 0.13 mmol) and $NaHCO_3$ (2 mg). The mixture was heated to 150 °C for 1 h and, after cooling to rt, poured into Et_2O (5 mL). The organic phase was evaporated and the residue purified by CC (hexane/EtOAc 1:1) to give the compound **13** (11 mg, 23%) as a white solid. TLC (hexane/EtOAc 1:2): R_f 0.05. ^1H NMR (300 MHz, $CDCl_3$): δ 1.74−2.03 (m, 6H, 1H-6′, 2H-7′, (CH_3)C-5), 2.63−2.65 (m, 1H, 1H-6′), 3.95 (dd, J = 10.5 Hz, 6.3 Hz 1H, H-5′), 4.49 (s, 2H, CH_2Ph), 4.62 (d, J = 11.4 Hz,1H, 1CH_2Ph), 4.69 (d, J = 11.4 Hz, 1H, 1CH_2Ph), 4.82 (d, J = 5.3 Hz, 1H, H-4′), 5.33 (d, J = 6.0 Hz, 1H, H-2′), 6.38 (d, J = 6.0 Hz, 1H, H-1′), 7.25−7.44 (m, 11H, 2Ph, H-6). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 25.3, 29.7, 67.2, 71.3, 85.5, 92.3, 93.0, 95.4, 119.0, 127.2, 127.6, 127.7, 128.1, 128.4, 128.7, 130.2, 137.1, 137.4, 159.7, 172.3. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{26}H_{27}O_5N_2$: 447.1920; found: 447.1914.

(3′S, 5′R)-1-(2′-hydroxy-3′,5′-O-dibenzyl-3′,5′-ethano-β-D-arabinofuranosyl)thymidine (14). A stirred solution of **13** (50 mg, 0.11 mmol) in 0.1 M KOH in EtOH (10 mL) was refluxed for 3 h. After cooling to rt, the mixture was neutralized with 1 M aq HCl and concentrated in vacuo. The residual aqueous solution was saturated with NaCl and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with sat aq $NaHCO_3$ (2 × 30 mL), dried over $MgSO_4$, and evaporated, and the residue waspurified by CC (hexane/EtOAc 1:2) to give the product **14** (17 mg, 32%) as a white foam. TLC (hexane/EtOAc 1:2): R_f 0.24. ^1H NMR (300 MHz, $CDCl_3$): δ 1.60 (sb, 4H, 2′−OH, (CH_3)C-5), 1.82 (m, 1H, H-7′), 2.10 (m, 1H, H-7′), 2.23 (m, 1H, H-6′), 2.46 (m, 1H, H-6′), 4.10−4.13 (m, 1H, H-5′), 4.38 (sb, 1H, H-2′), 4.43 (d, J = 4.7 Hz, 1H, H-4′), 4.46−4.72 (m, 4H, 2CH_2Ph), 6.30 (d, J = 2.5, 1H, H-1′), 7.28−7.34 (m, 10H, Ph), 7.46 (d, J = 0.8 Hz, 1H, H-6), 9.06 (sb, 1H, NH). ^1H NMR-NOE (400 MHz, $CDCl_3$): 6.30 (H-1′) → 4.38 (H-2′, 3.99%), 4.31 (H-4′, 1.41%). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.2, 14.2, 21.0, 23.8, 30.0, 60.4, 67.3, 71.6, 72.2, 78.9, 88.3, 89.7, 95.5, 108.7, 127.4, 127.8, 127.9, 128.4, 128.5, 128.9, 136.8, 137.9, 138.0, 150.0, 164.1. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{26}H_{29}O_6N_2$ 465.2026, found 465.2020.

(3′S,5′R)-1-(2′-O-Methylsulfonyl-3′,5′-O-dibenzyl-3′,5′-ethano-β-D-arabinofuranosyl)thymidine (12). To a stirred solution of **5** (0.3 g, 0.64 mmol) in pyridine (3.5 mL) was added dropwise MsCl (0.22 g, 1.9 mmol) at 0 °C. After 1 h at rt, the reaction was quenched with water (10 mL) and the mixture extracted with CH_2Cl_2 (3 × 15 mL). The combined organic phases were washed with satd aq $NaHCO_3$ (3 × 30 mL), dried over $MgSO_4$, and evaporated, and the residue was purified by CC (hexane/EtOAc 1:2) to give the product **12** (0.31 g, 89%) as a white solid. TLC (hexane−EtOAc 1:2): R_f 0.48. ^1H NMR (300 MHz, $CDCl_3$): δ 1.48 (d, J = 1.1 Hz, 3H, CH_3), 2.20−2.26 (m, 4H, 2H- 6′, 2H- 7′), 3.01 (s, 3H, CH_3), 4.06 (m, 1H, H-5′), 4.55−4.84 (m, 5H, 2CH_2Ph, H-4′), 5.02 (d, J = 7.9 Hz, 1H, H-2′), 6.47 (d, J = 7.9 Hz, 1H, H-1′), 7.28−7.37 (m, 10H, Ph), 7.54 (d, J = 1.3 Hz, 1H, H-6), 8.48 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.0, 28.5, 29.6, 38.5, 67.8, 71.4, 76.6, 77.8, 82.1, 86.7, 87.4, 88.3, 112.1, 126.9, 127.65, 127.67, 128.1, 128.5, 128.7, 135.0, 137.4, 138.3, 150.5, 163.2. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{27}H_{31}O_8N_2S$ 543.1801, found 543.1796.

(3′S,5′R)-1-(2′-Hydroxy-3′,5′-O-dibenzyl-3′,5′-ethano-β-D-arabinofuranosyl)thymidine (14) (from Compound 12). To a stirred solution of **12** (0.30 g, 0.55 mmol), in EtOH (10 mL) and H_2O (10 mL) was added 1 M aq NaOH (2.2 mL), and the reaction mixture was stirred under reflux for 16 h. After being cooled to rt, the mixture was neutralized with 1 M aq HCl and concentrated in vacuo. The resulting aqueous solution was saturated with NaCl and then extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with sat aq $NaHCO_3$ (2 × 30 mL), dried over $MgSO_4$, and evaporated, and the residue was purified by CC (hexane/EtOAc, 1:2) to give the product **14** (0.22 g, 85%) as a white foam. Analytical data identical as described above.

(3′S,5′R)-1-(2′-O-tert-Butydimethylsilyl-3′,5′-O-dibenzyl-3′,5′-ethano-β-D-arabinofuranosyl)thymidine (15). To a solution of nucleoside **14** (150 mg, 0.32 mmol) in 1 mL of DMF were added imidazole (55 mg, 0.8 mmol) and TBS-OTf (126 mg, 0.48 mmol). After being stirred at rt for 20 h, the mixture was diluted with CH_2Cl_2 (20 mL) and washed with water (15 mL) and brine (15 mL). The organic phase was dried over $MgSO_4$ and evaporated and the residue purified by CC (hexane/EtOAc 3:2) to give **15** (0.13 g, 71%) as a white foam. TLC (hexane/EtOAc 1:2): R_f 0.41 ^1H NMR (300 MHz, $CDCl_3$): δ −0.14 (s, 3H, Si-CH_3), 0.15 (s, 3H, Si-CH_3), 0.87 (s, 9H, t-Bu), 1.57−1.65 (m, 1H, 1H-7′), 1.83 (s, 3H, (CH_3) C-5), 1.90−2.08 (m, 2H, 1H-6′, 1H-7′), 2.26−2.30 (m, 1H, H-6′), 3.97−4.01 (m, 1H, H-5′), 4.31 (d, J = 5.3 Hz, 1H, H-4′), 4.37 (d, J = 3.6 Hz, 1H, H-2′), 4.43 (d, J = 11.1 Hz, 1H, CH_2Ph), 4.48 (d, J = 11.1 Hz, 1H, CH_2Ph), 4.54 (d, J = 11.5 Hz, 1H, CH_2Ph), 4.69 (d, J = 11.5 Hz, 1H, CH_2Ph), 4.53 (d, J = 11.5 Hz, 1H, CH_2Ph), 4.67 (d, J = 11.5 Hz, 1H, CH_2Ph), 6.15 (d, J = 3.6 Hz, 1H, H-1′), 7.28−7.48 (m, 10H, Ph), 7.48 (d, J = 1.32 Hz, 1H, H-6), 8.26 (s, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.2, −4.9, −3.6, −3.0, 12.4, 17.9, 25.6, 25.7, 25.8, 26.5, 30.8, 66.7, 71.7, 75.4, 77.8, 84.3, 86.1, 95.0, 109.0, 127.2, 127.9, 128.46, 128.54, 137.6, 138.0, 139.0, 149.9, 163.3. HRMS (ESI): m/z [M + Na]$^+$ calcd for $C_{32}H_{42}O_6N_2NaSi$ 601.2710, found 601.2704.

(3′S,5′R)-1-[3′,5′-Dihydroxy-2′-O-(tert-butyldimethylsilyl)-3′,5′-ethano-β-D-arabinofuranosyl]thymidine (16). To a solution of **15** (50 mg, 0.086 mmol) in EtOAc (2 mL) were added 20% $Pd(OH)_2$/C (50 mg) and 1,3-cyclohexadiene (69 mg, 0.86 mmol). The mixture was flushed with Ar for 15 min and set under an atmosphere of H_2. After being stirred for 2 h, the mixture was filtered through a pad of Celite, and the solvents were removed under reduced pressure. The residue was purified by CC (hexane/EtOAc 1:3) to give the title compound **16** (20 mg, 58%) as a white solid. TLC (hexane/EtOAc 1:3): R_f 0.16. ^1H NMR (300 MHz, MeOD): δ −0.28 (s, 3H, Si-CH_3), 0.01 (s, 3H, Si-CH_3), 0.75 (s, 9H, t-Bu), 1.15−1.27 (m, 1H, 1H-7′), 1.60−1.69 (m, 1H, H-6′), 1.72 (s, 3H, (CH_3)C-5), 1.79−1.90 (m, 1H, 1H-6′), 2.13−2.21 (m, 1H, 1H-7′), 3.78 (d, J = 5.1 Hz, 1H, H-4′), 3.96 (d, J = 3.6 Hz, 1H, H-2′), 3.97−4.05 (m, 1H, H-5′), 5.90 (d, J = 3.6 Hz, 1H, H-1′), 7.57 (d, J = 1.1 Hz, 1H, H-6). ^{13}C NMR (75 MHz, MeOD): δ −4.9, −4.7, 12.7, 19.1, 26.7, 32.2, 33.7, 73.5, 81.6, 87.4, 89.0, 89.6, 109.9, 141.3, 152.3, 166.7. HRMS (ESI): m/z [M + H]$^+$ calcd for $C_{18}H_{31}O_6N_2Si$ 399.1951, found 399.1946.

(3′S,5′R)-1-[3′-Hydroxy-5′-O-(4,4′-dimethoxytriphenylmethyl)-2′-O-(tert-butyldimethylsilyl)-3′,5′-ethano-β-D-

arabinofuranosyl]thymidine (17). To a stirred solution of nucleoside **16** (0.24 g, 0.6 mmol) in pyridine (3 mL) was added DMTr-Cl (0.60 g, 1.8 mmol) at rt. After 5 h, the reaction was quenched with satd aq $NaHCO_3$ (5 mL), and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic phases were dried over $MgSO_4$, filtered, and evaporated, and the residue was purified by CC (hexane/EtOAc 2:1 + 1% Et_3N) to give the title compound **17** (0.38 g, 90%) as a white solid. TLC (hexane/EtOAc 1:2 +1%Et_3N): R_f 0.47. ^{1}H NMR (300 MHz, $CDCl_3$): δ = −0.13 (s, 3H, Si-CH_3), 0.15 (s, 3H, Si-CH_3), 0.89 (s, 9H, *t*-Bu), 1.07−1.10 (m, 1H, 1H-7′), 1.47−1.51(m, 1H, 1H-6′), 1.61−1.78 (m, 1H, 1H-6′), 1.94 (s, 3H, (CH_3)C-5, 2.04−2.19 (m, 1H, 1H-7′), 2.23 (bs, 1H, OH), 3.19 (d, *J* = 5.3 Hz, 1H, H-4′), 3.77 (s, 6H, 2OMe), 3.95−3.98 (m, 1H, H-5′), 4.08 (d, *J* = 3.9 Hz, 1H, H-2′), 6.00 (d, *J* = 3.9 Hz, 1H, H-1′ (sb, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.0, −4.8, 12.9, 18.2, 26.1, 30.0, 31.2, 31.9, 55.5, 74.1, 80.7, 85.6, 86.9, 87.0, 88.6, 109.3, 113.4, 127.2, 128.1, 128.5, 130.51, 130.54, 137.11, 137.13, 139.3, 146.0, 150.4, 158.9, 163.7. HRMS (ESI): *m*/*z* $[M + Na]^+$ calcd for $C_{39}H_{48}O_8N_2NaSi$ 723.3078, found 723.3072.

(3′*S*,5′*R*)-1-[3′-*O*-(Cyanoethoxydiisopropylaminophosphino)-5′-*O*-[(4,4′-dimethoxytriphenyl)methyl]-2′-*O*-(*tert*-butyldimethylsilyl)-3′,5′-ethano-*β*-D-arabinofuranosyl]thymidine (18). To a stirred solution of **17** (0.34 g, 0.49 mmol) in MeCN (3 mL) were added $^iPr_2NEt$ (0.3 g, 2.4 mmol) and $^iPr_2NP(Cl)OCH_2CH_2CN$ (0.32 g, 1.4 mmol) at rt. After 1 h, the mixture was diluted with EtOAc (10 mL) and washed with satd aq $NaHCO_3$ (2 × 10 mL). The aqueous phases were extracted with EtOAc (3 × 10 mL) and the combined organic phases dried over $MgSO_4$ and evaporated, and the residue was purified by CC (hexane/EtOAc 1:2 + 1% Et_3N) to give **18** (0.43 g, 97%) as a white foam. TLC (hexan/EtOAc 2:1 + 1% Et_3N): R_f 0.63. ^{1}H NMR (300 MHz, $CDCl_3$): δ −0.162, −0.168, 0.09, 0.10 (4s, 6H, Si-CH_3), 0.89 (s, 9H, ^{t}Bu), 1.06−1.15 (m, 12H, 4N−CH_3), 1.15- 1.78, (m, 3H, 2H-6′, 1H-7′), 1.95 (s, 3H, (CH_3)C-5, 2.04−2.12 (m, 1H, 1H-7), 2.54−2.56 (m, 2H, CH_2CN), 3.45−3.65 (m, 4H, CH_2−O, 2CH-N), 3.80 (s, 6H, 2OMe), 3.90 (m, 1H, H-5′), 4.29−4.32 (m, 1H, H-4′), 5.91, 5.95 (2d, J = 3.4 Hz, 1H, H-1′), 6.79−6.84 (m, 4H, Ph), 7.15−7.61 (m, 10H, Ph, H-6), 8.30−8.50 (m, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ −5.3, −5.2, −5.1, −4.99, −4.95, −4.90, 12.6, 14.2, 17.9, 20.08, 20.14, 22.7, 24.1, 24.18, 24.24, 24.3, 24.4, 24.5, 25.8, 29.5, 29.6, 31.8, 32.0, 43.2, 43.3, 43.4, 43.5, 55.2, 55.3, 57.7, 57.9, 73.2, 73.3, 79.0, 79.1, 79.3, 85.4, 85.5, 85.6, 85.9, 86.0, 86.63, 86.65, 92.4, 92.45, 92.48, 92.6, 108.7, 108.8, 113.1, 117.5, 126.8, 127.8, 128.2, 130.2, 130.3, 136.8, 136.9, 138.98, 139.03, 145.6, 149.96, 149.99, 156.6, 163.5. ^{31}P NMR (162 MHz, $CDCl_3$): δ 140.96, 142.13. HRMS (ESI): *m*/*z* $[M + H]^+$ calcd for $C_{48}H_{66}O_9N_4$ PSi 901.4337, found 901.4331.

Oligonucleotide Synthesis and Purification. The synthesis of oligonucleotides was performed either on a 1.3 μmol scale with a Pharmacia LKB Gene Assembler Special DNA-synthesizer or on a 1 μmol scale with a Polygen DNA synthesizer by using standard phosphoramidite chemistry. The phosphoramidite building blocks of the natural nucleosides and the nucleosides bound to CPG-solid support were purchased from Glen Research or Vivotide. Solvents and reagents used for the synthesis were prepared according to the indications of the manufacturer. 5-(Ethylthio)-1*H*-tetrazole (ETT) was used as an activator, and 3% dichloroacetic acid in dichloroethane was used for detritylation. The concentrations for the natural phosphoramidite solutions were 0.1 M and for the modified phosphoramidites 0.15 or 0.2 M. The coupling times for natural phosphoramidites were 1.5 min and for the modified phosphoramidite 12−14 min. The coupling efficiencies for **11** and **18** were generally low (~90%) as judged from the trityl assay. Deprotection and detachment from solid support were performed in concentrated NH_3/EtOH (3:1, 0.5 mL, 55 °C, 30 h). Removal of the silyl groups was performed by treatment of the crude oligonucleotides with 1 M TBAF in THF (0.5 mL) at rt for 24 h. After evaporation, the brown residue was taken up in H_2O and filtered through a Sep-Pak C-18 cartridge (Waters). The crude samples were purified by ion exchange HPLC (Dionex, DNAPac-200, 4.6 × 250 mm column with precolumn). The product containing fractions were concentrated and again desalted over Sep-Pak C-18 cartridge (Waters) according to the protocol of the manufacturer. The following buffers were used for HPLC: A: 25 mM Trizma base in H_2O, pH 8.0; B: 25 mM Trizma Base, 1.25 M NaCl, in H_2O, pH 8.0. Linear gradients of B in A were used. The integrity of all oligonucleotides was confirmed with ESI^--MS (see the Supporting Information).

Melting Curves. Thermal denaturation experiments were carried out on a Varian Cary 100 Bio UV/vis spectrophotometer. Absorbances were monitored at 260 nm, and the heating rate was set to 0.5 °C min^{-1}. A cooling−heating−cooling cycle in the temperature range of 80 −15 °C was applied. The first derivative of the melting curves were calculated with the Varian WinUV software. To avoid evaporation of solvents, a layer of dimethylpolysiloxane was added over the samples within the cell. All measurements were carried out in standard saline buffer (150 mM NaCl, 10 mM Na_2HPO_4, pH 7.0) at a total oligonucleotide concentration of 2 μM.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H, ^{13}C, and ^{31}P NMR spectra of compounds **2−18**, analytical data of oligonucleotides, and X-ray structural data and packing diagram of compound **5**. This material is available free of charge via the Internet at http://pubs.acs.org/.

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