Synthesis of a Novel Bicyclic Nucleoside Restricted to an *S*-Type Conformation and Initial Evaluation of Its Hybridization Properties When Incorporated into Oligodeoxynucleotides

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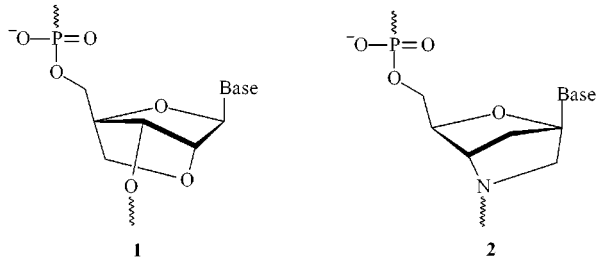
The phosphoramidite (1*S*,3*R*,4*S*)-3-(2-cyanoethoxy(diisopropylamino)phosphinoxymethyl)-5-*N*-(4-monomethoxytrityl)-1-(uracil-1-yl)-5-aza-2-oxabicyclo[2.2.1]heptane **18** of a novel bicyclic nucleoside structure was synthesized from the known 1-(3′-deoxy-β-D-psicofuranosyl)uracil **3**. Conformational analysis of its structure verified its expected *S*-type furanose conformation, and the secondary amino group in the 4′-position allowed for incorporation into oligonucleotides using 5′ → 3′ directed oligonucleotide synthesis as previously described for phosphoramidates. Thermal denaturation studies showed rather large decreases in duplex stabilities of −4.3 and −2.7 °C per modification toward complementary DNA and RNA, respectively.

Introduction

Locked nucleic acid (LNA) **1** (Figure 1) is a novel nucleic acid analogue that displays unprecedented binding affinity toward complementary DNA and RNA.[1−4] The 2′,4′-oxymethylene bridge efficiently restricts the furanose ring into an *N*-type conformation as verified by X-ray studies of monomers[5] and NMR studies of duplexes.[6,7] An interesting study concerns the corresponding novel 3′,1′-aminomethylene linked counterpart **2**, which is believed to be restricted just as efficiently into an *S*-type furanose conformation. The trivalent amino group allows for incorporation into oligonucleotides by a 5′ → 3′ directed variation of the standard phosphoramidite approach for synthesis of oligonucleotides.[8−10] Though



1 **2**

Figure 1. The general structure of LNA (**1**) depicted in its *N*-type furanose conformation compared to the novel 1′,3′-linked counterpart (**2**) depicted in its expected *S*-type furanose conformation verified herein.

N-type conformations leading to A-type duplexes generally effect the highest duplex stabilities,[11−13] an interesting feature of a restricted *S*-type conformation is the possibility of activating RNase H, the enzyme that cleaves the RNA strand in RNA/DNA heteroduplexes, thus allowing the modified oligonucleotide to target multiple RNA strands. RNase H is believed to recognize duplexes intermediate between the A- and B-forms,[14−17] which favors oligonucleotide analogues capable of resembling natural DNA with its *S*-type furanose conformations, as also seen for arabino nucleic acids[18] and cyclohexene nucleic acids.[19]

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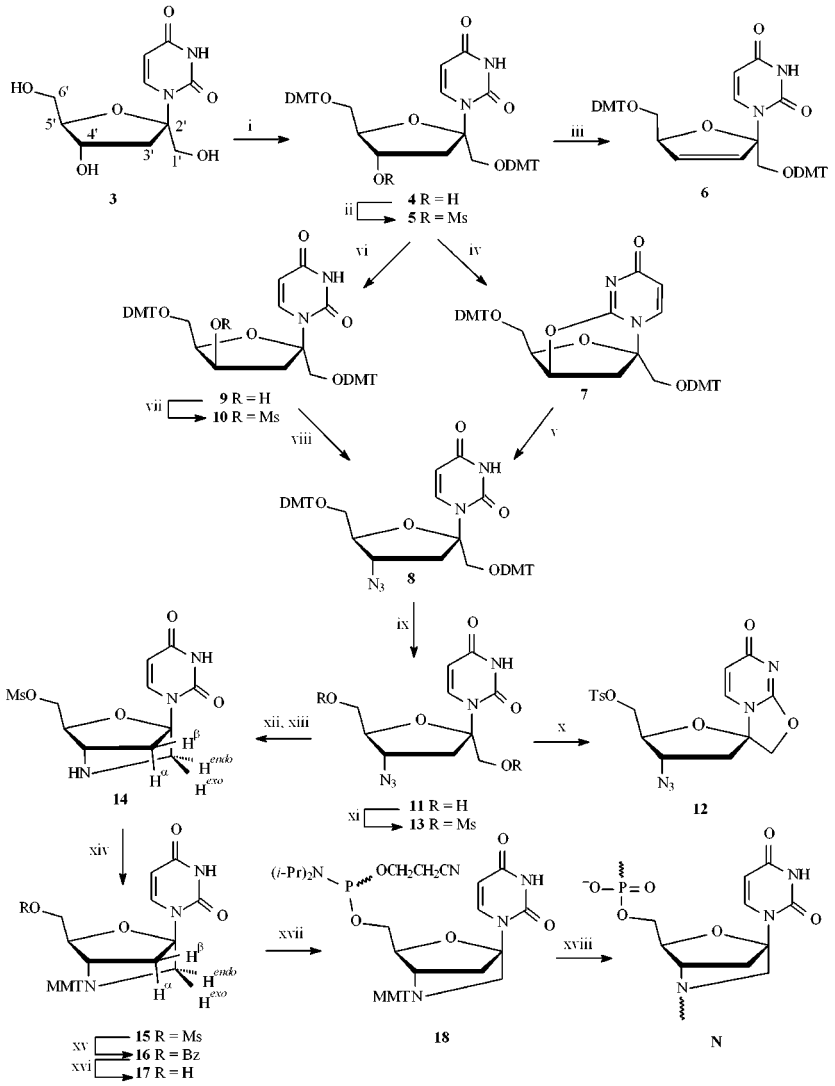
Scheme 1[a]



[a] Reagents and conditions: (i) DMTCl, pyridine, CH_2Cl_2, 77%; (ii) MsCl, pyridine, 93%; (iii) NaH (2 equiv), DMF, 92%; (iv) NaH (1.06 equiv), DMF, 80%; (v) NaN_3, DMF, 26%; (vi) 1 M NaOH (aqueous), 1,4-dioxane, 93% (2 steps from **4**); (vii) MsCl, pyridine; (viii) NaN_3, DMF, 97% (2 steps); (ix) 80% aqueous AcOH, 81%; (x) TsCl, pyridine, CH_2Cl_2, 65%; (xi) MsCl, pyridine, 92%; (xii) H_2, 10% Pd/C, MeOH; (xiii) DMF, 60 °C, 75% (2 steps); (xiv) MMTCl, pyridine, 80%; (xv) NaOBz, DMF; (xvi) NaOMe, MeOH, 78% (2 steps); (xvii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 73%. DMT = 4,4′-dimethoxytrityl, MMT = 4-monomethoxytrityl; (xviii) DNA synthesizer.

Results and Discussion

Starting from the known 1-(3′-deoxy-β-D-psicofuranosyl)uracil **3**[20,21] with the desired carbon skeleton already present (Scheme 1), a 4,4′-dimethoxytrityl (DMT) protection of the primary hydroxy groups using DMTCl in pyridine and CH_2Cl_2 proceeded in 77% yield. Introduction of a correctly configured nitrogen atom in the 4′-position (refer to structure **3**, Scheme 1 for numbering used throughout the discussion) was initially thought to be possible via the anhydronucleoside **7**. Mesylation of the secondary hydroxy group of **4** using MsCl in pyridine at 0 °C yielded nucleoside **5** in 93% yield, while concomitant treatment with 2 equiv of NaH in DMF resulted in an almost quantitative elimination to the unsaturated nucleoside **6**. By contrast, a similar treatment with only 1.06 equiv of NaH in DMF afforded the expected anhydro structure **7** in 80% yield. Nucleophilic opening of the anhydro bond with inversion of stereochemistry to give azide **8** proceeded in only 26% yield using NaN_3 in DMF at 120 °C, while yields were even lower using LiN_3 under similar conditions. Alternatively, inversion of the configuration at C4′ before introduction of a nitrogen atom was investigated. Without purification, the crude mesylate **5** was reacted with 1 M NaOH (aqueous) in dioxane at 90 °C to give compound **9** in 93% yield. The inversion of configuration at C4′ is believed to result from the nucleophilic attack of hydroxide ions at the nucleobase C2 of the intermediary anhydro structure **7**. After mesylation of **9** using MsCl in pyridine at room temperature, introduction of the azide substituent by an S_N2 displacement to give **8** was accomplished in 97% yield (from **9**) by direct treatment of crude **10** with NaN_3 in DMF at 70

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°C. Nucleoside **11** (devoid of anti HIV-1 activity when tested as described in ref 22) was obtained in 81% yield by treatment with 80% AcOH, while tosylation using TsCl in pyridine and CH_2Cl_2 afforded the 2,1′-anhydronucleoside **12** in only 65% yield after prolonged reaction time. Alternatively, mesylation of **11** with MsCl in pyridine at 0 °C instead gave the expected mesylate **13** in 92% yield, and the following reduction of the azide was accomplished by catalytic hydrogenation using 10% Pd/C in MeOH under an atmosphere of hydrogen. As a result of the formation of a more polar byproduct believed to be the 2,1′-anhydronucleoside of the intermediary primary amine, purification was not performed until after cyclization to the bicyclic structure **14**, which was isolated in 75% yield (from **13**) after heating to 60 °C in DMF. To prepare the aminonucleoside for 5′ → 3′ directed oligonucleotide synthesis[8−10] while still using the standard diisopropylamino substituent on phosphorus, the amino group was derivatized with a 4-monomethoxytrityl (MMT) group[8] by treatment with MMTCl in pyridine, which afforded nucleoside **15** in 80% yield. Simple basic hydrolysis of the mesyl protecting group using aqueous NaOH in dioxane at elevated temperatures proved troublesome, while reflux with KOH in EtOH resulted only in ethoxide substitution. Alternatively, a two-step sequence was applied in which treatment with NaOBz in DMF at 100 °C afforded the intermediate benzoate **16**, which was debenzoylated with NaOMe in MeOH to give nucleoside **17** in 78% yield (from **15**). Finally, 6′-O-phosphitylation using 2-cyanoethyl-*N,N*-diisopropylphosphoramidochlorodite and *N,N*-diisopropylethylamine in CH_2Cl_2 furnished the phosphoramidite **18** in 73% yield.

Conformational analysis of the bicyclic nucleoside analogues **14**−**17** by NOE difference spectroscopy supported the assumption of a rigid structure with the furanose ring restricted to an *S*-type conformation and the nucleobase in the normal *anti* conformation. Thus, irradiation of proton signals proved H3′$_\beta$ and H6 (NOEs of 1−3%), as well as H3′$_\beta$ and H6′ (NOEs of 2−3%), to be mutually close, and the proximity of H3′$_\alpha$ and H1′$_{exo}$ (NOEs of ∼2%) was likewise verified. Values of $^3J_{3'\alpha,4'}$ and $^3J_{3'\beta,4'}$ in the ranges of 1.7−2.1 and 0 Hz, respectively, also correspond to a pseudorotation angle P in the southern part of the pseudorotation circle generally used to describe furanose conformations.[23]

Phosphoramidite **18** was incorporated three times into the mixed 9-mer oligonucleotide sequence depicted in Table 1 (entry 3) by 5′ → 3′ directed oligonucleotide synthesis using commercial "inverse" 3′-*O*-DMT-5′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-2′-deoxyadenosine, -2′-deoxycytidine and -2′-deoxyguanosine amidites as the other building blocks. Standard conditions were used except for a 10 min coupling time for the inverse amidites (refer to Experimental Section for further details) and 12 min for the amidite **18**. Initially, the modified sequence was synthesized in the DMT-*ON* mode and, after deblocking and cleavage from the solid support, purified by reversed phase chromatography (Cruachem disposable cartridges) yielding a product of >90% purity as judged from capillary gel electrophoresis recorded immediately after the purification. After evaporation of the solvent and dissolution in pure water, no verification of the composition could be obtained by MALDI-MS analysis, and capillary gel electrophoresis at this stage also proved the sequence to be fully degraded. This can be ascribed to the apparently general susceptibility of bicyclic phosphoramidates toward acidic hydrolysis even in almost neutral solutions.[24] Alternatively, the resynthesized sequence was purified by anion-exchange FPLC at pH 12, and after desalting capillary gel electrophoresis showed >90% purity of the product, the composition of which could also be verified from MALDI-MS analysis.

Table 1. Sequences Synthesized and Thermal Denaturation Studies toward Complementary DNA and RNA Sequences[a]

		complementary DNA		complementary RNA	
entry	sequence	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)
1	5′-d(GTGATATGC)	29	+0.7	27	+0.3
2	5′-d(G**U**GA**U**A**U**GC)	27		26	
3	5′-d(G**N**GA**N**A**N**GC)	14	−4.3	18	−2.7

[a] T_m values measured as the maximum of the first derivative of the melting curve (A_{260} vs temperature) recorded in medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 μM concentrations of the two complementary strands. ΔT_m = change in T_m value calculated *per modification*. dA= 2′-deoxyadenosine monomer, dC = 2′-deoxycytidine monomer, dG = 2′-deoxyguanosine monomer, dU = 2′-deoxyuridine monomer, dT = thymidine monomer, **N** = **2** (Base = U, see Figure 1); all abbreviated as d(sequence).

As depicted in Table 1, the melting temperature (T_m value) and the change in T_m value per modification compared with the unmodified reference containing 2′-deoxyuridine monomers in place of the bicyclic amine were determined. As can be seen when comparing entry 1 with entry 2, a slight decrease in the thermal stability (−0.7 °C per modification toward DNA and −0.3 °C per modification toward RNA) results from substituting the natural thymine bases with uracil. Unfortunately, a much larger decrease of −4.3 °C per modification toward DNA and −2.7 °C per modification toward RNA was observed for the modified sequence containing three bicyclic monomers (entry 3). This rather large decrease can be attributed to the rigid bicyclic nucleoside structure, which obviously suffers from unfavorable sterical or conformational restrictions, at least in a context of unmodified 2′-dexoynucleotide monomers.

Conclusion

In conclusion, a novel bicyclic nucleoside structure has been synthesized with a uracil nucleobase. The introduction of nitrogen at C4′ was successfully accomplished by inversion of the configuration at C4′ prior to nucleophilic substitution by azide. Conformational analysis by NOE difference spectroscopy verified the furanose ring of the rigid nucleoside to be restricted into an *S*-type conformation. The phosphoramidite derivative **18** was incorporated three times into a mixed 9-mer oligonucleotide by 5′ → 3′ directed oligonucleotide synthesis using commercial inverse phosphoramidites (3′-*O*-DMT-5′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-2′-deoxynucleosides). Thermal denaturation studies revealed rather large decreases in duplex stabilities when hybridized to complementary DNA and RNA.

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Experimental Section

General. Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. All reagents were obtained from commercial suppliers and were used without further purification. Petroleum ether of distillation range 60−80 °C was used. The silica gel (0.040−0.063 mm) used for column chromatography was purchased from Merck. After column chromatography, fractions containing product were pooled, evaporated under reduced pressure, and dried overnight under high vacuum to give the product. All ^{1}H NMR spectra were recorded at 400 MHz, all ^{13}C NMR spectra were recorded at 100.6 MHz, and the ^{31}P spectrum was recorded at 121.5 MHz. Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as internal standard for ^{1}H and ^{13}C and relative to 85% H_3PO_4 as external standard for ^{31}P. Assignments of NMR spectra are based on 2D spectra and follow standard carbohydrate/nucleoside nomenclature (i.e., the furanose skeleton numbered 1′ to 6′, see Scheme 1) even though the systematic compound names of the bicyclic structures are given according to the von Baeyer nomenclature. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode, and microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

1-[3′-Deoxy-1′,6′-di-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]uracil (4).[20,21] Triol **3** (2.711 g, 10.5 mmol) was dissolved in anhydrous pyridine (50 mL) at 0 °C, whereafter 4,4′-dimethoxytrityl chloride (7.823 g, 23.0 mmol) dissolved in anhydrous CH_2Cl_2 (150 mL) was added dropwise during 3 h. After the end of addition, the mixture was allowed to heat slowly to room temperature. After 16 h at room temperature, the mixture was cooled to 0 °C, additional 4,4′-dimethoxytrityl chloride (3.032 g, 8.92 mmol) was added portionwise during 1.5 h, and the mixture was allowed to heat slowly to room temperature. After 21 h, MeOH (5 mL) and EtOAc (400 mL) were added, and the mixture was washed successively with saturated aqueous $NaHCO_3$ (50 mL) and H_2O (50 mL). The organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (2 × 50 mL). The residue was purified by silica gel column chromatography (18 × 5.5 cm) eluting with a gradient of 0.5:0.5−2:99−97.5 pyridine/MeOH/CH_2Cl_2 (v/v/v) to give compound **4** (2.711 g, 77%) as a white foam after coevaporation with acetonitrile (3 × 50 mL): R_f (7% MeOH in CH_2Cl_2 (v/v)) 0.59; ^{1}H NMR ($CDCl_3$) δ 8.65 (1H, bs, NH), 7.79 (1H, d, J = 8.3 Hz, H6), 7.38−7.18 (18H, m, DMT), 6.83−6.77 (8H, m, DMT), 5.43 (1H, dd, J = 2.2, 8.2 Hz, H5), 4.39 (1H, m, H5′), 4.20 (1H, m, H4′), 3.75 (6H, s, OMe), 3.75 (6H, s, OMe), 3.68 (1H, d, J = 9.4 Hz, H1′), 3.40 (1H, m, OH), 3.37 (1H, d, J = 9.7 Hz, H1′), 3.13 (1H, dd, J = 4.9, 10.7 Hz, H6′), 3.09 (1H, dd, J = 5.5, 10.7 Hz, H6′), 2.71 (1H, dd, J = 6.0, 15.4 Hz, H3′), 2.61 (1H, d, J = 15.4 Hz, H3′); ^{13}C NMR ($CDCl_3$) δ 163.47 (C4), 158.67, 158.60 (DMT), 149.49 (C2), 144.28, 143.65 (DMT), 141.16 (C6), 135.29, 135.28, 134.92, 134.89, 129.90, 129.87, 128.31, 127.93, 127.87, 127.83, 127.17, 126.98, 113.23, 113.16 (DMT), 100.46 (C5), 99.23 (C2′), 89.01 (C5′), 87.92, 86.73 (Ar_2PhC), 73.02 (C4′), 66.07 (C1′), 63.33 (C6′), 55.20, 55.18 (OMe), 44.43 (C3′).

1-[3′-Deoxy-1′,6′-di-*O*-(4,4′-dimethoxytrityl)-4′-*O*-methanesulfonyl-β-D-psicofuranosyl]uracil (5). Compound **4** (2.725 g, 3.16 mmol) was dissolved in anhydrous pyridine (20 mL) and cooled to 0 °C, methanesulfonyl chloride (0.75 mL, 9.7 mmol) was added, and the mixture was stirred for 2 h. Saturated aqueous $NaHCO_3$ (50 mL) and H_2O (50 mL) were added, and the mixture was extracted with EtOAc (4 × 50 mL). The combined organic phase was washed with H_2O (2 × 40 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (2 × 40 mL). The residue was purified by silica gel column chromatography (8.5 × 5.5 cm) eluting with a gradient of 0.5:5−10:94.5−84.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) to give compound **5** (2.770 g, 93%) as a white foam after coevaporation with anhydrous acetonitrile (3 × 40 mL) and CH_2Cl_2 (30 mL): R_f (1:3 EtOAc/CH_2Cl_2 (v/v)) 0.60; ^{1}H NMR ($CDCl_3$) δ 8.61 (1H, d, J = 1.9 Hz, NH), 7.89 (1H, d, J = 8.4 Hz, H6), 7.35−7.18 (18H, m, DMT), 6.83−6.77 (8H, m, DMT), 5.61 (1H, dd, J = 2.4, 8.4 Hz, H5), 4.97 (1H, d, J = 6.2 Hz, H4′), 4.45 (1H, bs, H5′), 3.77 (6H, s, OMe), 3.75 (3H, s, OMe), 3.75 (3H, s, OMe), 3.64 (1H, d, J = 10.1 Hz, H1′), 3.42 (1H, d, J = 10.1 Hz, H1′), 3.31 (1H, dd, J = 3.9, 10.8 Hz, H6′), 3.25 (1H, dd, J = 4.8, 10.8 Hz, H6′), 2.89 (1H, dd, J = 6.5, 16.0 Hz, H3′), 2.81 (3H, s, Ms), 2.70 (1H, d, J = 16.0 Hz, H3′); ^{13}C NMR ($CDCl_3$) δ 163.31 (C4), 158.60, 158.41 (DMT), 149.33 (C2), 144.29, 143.92 (DMT), 142.16 (C6), 135.34, 135.31, 134.90, 134.85, 129.87, 129.81, 129.77, 127.86, 127.70, 127.67, 126.99, 126.76, 113.18, 113.02 (DMT), 100.09 (C5), 98.70 (C2′), 86.97, 86.46 (Ar_2PhC), 85.34 (C5′), 80.39 (C4′), 65.35 (C1′), 62.41 (C6′), 55.13, 55.08 (OMe), 42.08 (C3′), 38.29 (Ms).

1-[3′,4′-Didehydro-3′,4′-dideoxy-1′,6′-di-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]uracil (6). Compound **5** (880 mg, 0.935 mmol) dissolved in anhydrous DMF (7.5 mL) was added to a suspension of NaH (77 mg, 60% w/w, 1.9 mmol) in anhydrous DMF (3 mL) at 0 °C. After stirring for 17 h at room temperature, H_2O (50 mL) was added, and the mixture was extracted with EtOAc (30 mL), CH_2Cl_2 (2 × 30 mL), and EtOAc (2 × 30 mL). The combined organic phase was washed successively with saturated aqueous $NaHCO_3$ (50 mL) and H_2O (50 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (50 mL). The residue was purified by silica gel column chromatography (13 × 3.3 cm) eluting with a gradient of 0.5:5−10:94.5−89.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) to give compound **6** (727 mg, 92%) as a white foam after coevaporation with acetonitrile (4 × 15 mL) and CH_2Cl_2: R_f (5% MeOH in CH_2Cl_2 (v/v)) 0.60; FAB-MS m/z 844.8 $[M]^+$; ^{1}H NMR ($CDCl_3$) δ 8.94 (1H, bs, NH), 7.75 (1H, d, J = 8.2 Hz, H6), 7.37−7.15 (18H, m, DMT), 6.82−6.77 (8H, m, DMT), 6.69 (1H, dd, J = 2.2, 6.0 Hz, H3′), 6.14 (1H, dd, J = 1.5, 6.0 Hz, H4′), 5.44 (1H, dd, J = 2.3, 8.2 Hz Hz, H5), 5.12 (1H, bs, H5′), 3.76 (3H, s, OMe), 3.76 (3H, s, OMe), 3.74 (6H, 2s, OMe), 3.56 (1H, d, J = 9.7 Hz, H1′), 3.46 (1H, d, J = 9.7 Hz, H1′), 3.26−3.21 (2H, m, H6′); ^{13}C NMR ($CDCl_3$) δ 163.66 (C4), 158.42, 158.41, 158.35 (DMT),149.50 (C2), 144.39, 144.30 (DMT), 141.62 (C6), 135.50, 135.47, 135.40, 135.30 (DMT), 131.82 (C4′), 129.86, 129.83, 129.33 (DMT), 128.23 (C3′), 127.92, 127.78, 127.68, 127.63, 126.78, 126.68, 113.01, 112.96 (DMT), 103.45 (C2′), 100.11 (C5), 87.09 (C5′), 86.14, 85.99 (Ar_2PhC), 65.47 (C1′), 64.50 (C6′), 55.08, 55.03 (OMe).

1-[(2,4′-Anhydro)-3′-deoxy-1′,6′-di-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]uracil (7). Compound **5** (2.770 g, 2.94 mmol) was dissolved in anhydrous DMF (25 mL), NaH (125 mg, 60% (w/w), 3.12 mmol) was added, and the mixture was stirred at room temperature for 3 days. EtOAc (200 mL) was added, and the mixture was washed with H_2O (3 × 50 mL). The organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (2 × 50 mL). The residue was purified by silica gel column chromatography (7.8 × 5.5 cm) eluting first with 0.5:75:24.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) followed by 0.5:3:96.5 pyridine/MeOH/CH_2Cl_2 (v/v/v) to give anhydronucleoside **7** (1.978 g, 80%) as a white foam after coevaporation with acetonitrile (3 × 40 mL): R_f (7% MeOH in CH_2Cl_2 (v/v)) 0.44; FAB-MS m/z 845.8 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.39−7.16 (18H, m, DMT), 6.88−6.85 (4H, m, DMT), 6.80−6.76 (4H, m, DMT), 6.71 (1H, d, J = 7.8 Hz, H6), 5.78 (1H, d, J = 7.8 Hz, H5), 5.14 (1H, bs, H4′), 4.26 (1H, m, H5′), 3.80 (3H, s, OMe), 3.80 (3H, s, OMe), 3.76 (3H, s, OMe), 3.76 (3H, s, OMe), 3.76 (2H, s, H1′), 3.44 (2H, d, J = 6.5 Hz, H6′), 2.79 (1H, d, J = 12.2 Hz, H3′), 2.26 (1H, dd, J = 2.5, 12.8 Hz, H3′); ^{13}C NMR ($CDCl_3$) δ 170.52 (C4/C2), 158.90, 158.86, 158.41 (DMT), 153.85 (C2/C4), 144.25, 142.99 (DMT), 135.64 (C6), 135.25, 135.19, 134.01, 133.75, 129.87, 129.83, 129.76, 128.09, 127.78, 127.73, 127.71, 127.34, 126.74, 113.37, 113.08, 113.05 (DMT), 108.77 (C5), 93.79 (C2′), 87.19, 86.69 (Ar_2PhC), 85.15 (C5′), 76.98 (C4′), 61.89 (C6′), 61.39 (C1′), 55.17, 55.11 (OMe), 37.47 (C3′).

1-[4′-Azido-3′-deoxy-1′,6′-di-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]uracil (8). Anhydronucleoside **7** (481 mg, 0.569 mmol) was dissolved in anhydrous DMF (15 mL), NaN_3 (0.70 g, 11 mmol) was added, and the suspension was stirred at 120 °C for 23 h. EtOAc (150 mL) was added, and the mixture was washed successively with saturated aqueous $NaHCO_3$ (30 mL) and H_2O (2 × 30 mL). The combined aqueous phase was

extracted with EtOAc (2 × 30 mL), and the combined organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (4 × 40 mL). The residue was purified by silica gel column chromatography (15.3 × 3.3 cm) eluting first with a gradient of 0.5:0−10:99.5−89.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) followed by 0.5:4:95.5 pyridine/MeOH/CH_2Cl_2 (v/v/v) to give azide **8** (130 mg, 26%) as a yellowish foam after coevaporation with acetonitrile (4 × 15 mL), *n*-hexane/CH_2Cl_2 (2 × 15 mL, 4:1 (v/v)), and CH_2Cl_2 (2 × 15 mL): R_f (1:3 EtOAc/CH_2Cl_2 (v/v)) 0.64; IR (Nujol mull) ν 2106 cm^{-1} (strong); ^1H NMR ($CDCl_3$) δ 8.59 (1H, bs, NH), 7.89 (1H, d, J = 8.2 Hz, H6), 7.38−7.16 (18H, m, DMT), 6.85−6.77 (8H, m, DMT), 5.54 (1H, dd, J = 2.3, 8.2 Hz, H5), 4.08 (1H, m, H5′), 4.03 (1H, m, H4′), 3.78 (6H, s, OMe), 3.76 (3H, s, OMe), 3.75 (3H, s, OMe), 3.57 (1H, d, J = 10.1 Hz, H1′), 3.48 (1H, d, J = 10.1 Hz, H1′), 3.28 (2H, dd, J = 1.8, 4.2 Hz, H6′), 2.99 (1H, dd, J = 7.0, 14.9 Hz, H3′), 2.38 (1H, dd, J = 5.4, 14.8 Hz, H3′); ^{13}C NMR ($CDCl_3$) δ 163.36 (C4), 158.57, 158.40 (DMT), 149.42 (C2), 144.30, 144.12 (DMT), 142.03 (C6), 135.44, 135.31, 135.10, 129.81, 129.79, 127.89, 127.84, 127.77, 127.67, 126.96, 126.75, 113.15, 113.01, 112.99 (DMT), 100.17 (C5), 98.08 (C2′), 86.68, 86.35 (Ar_2PhC), 84.18 (C5′), 65.16 (C1′), 62.40 (C6′), 60.53 (C4′), 55.13, 55.07 (OMe), 39.98 (C3′).

1-[3′-Deoxy-1′,6′-di-*O*-(4,4′-dimethoxytrityl)-β-D-sorbofuranosyl]uracil (9). Compound **4** (4.353 g, 4.93 mmol) was dissolved in anhydrous pyridine (30 mL) and cooled to 0 °C, methanesulfonyl chloride (1.5 mL, 19.3 mmol) was added, and stirring was continued at 0 °C for 2 h. Saturated aqueous $NaHCO_3$ (100 mL) and H_2O (100 mL) were added, and the suspension was extracted with EtOAc (4 × 100 mL). The combined organic phase was washed with H_2O (2 × 80 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (2 × 100 mL). The crude residue **5** was dissolved in 1,4-dioxane (200 mL), 1 M NaOH (aqueous, 100 mL) was added, and the mixture was stirred at 90 °C for 10 h. After the mixture cooled, H_2O (200 mL) was added, and the suspension was extracted with EtOAc (4 × 125 mL). The combined organic phase was washed with saturated aqueous $NaHCO_3$ (80 mL) and H_2O (80 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (50 mL). The residue was purified by silica gel column chromatography (13.5 × 5.5 cm) eluting with a gradient of 0.5:0−3:99.5−96.5 pyridine/MeOH/CH_2Cl_2 (v/v/v) to yield compound **9** (3.935 g, 93%) as a white foam after coevaporation with acetonitrile (6 × 40 mL): R_f (5% MeOH in CH_2Cl_2 (v/v)) 0.25; FAB-MS m/z 863 $[M + H]^+$; ^1H NMR ($CDCl_3$) δ 8.75 (1H, bs, NH), 7.88 (1H, d, J = 8.2 Hz, H6), 7.52−7.15 (18H, m, DMT), 6.86−6.76 (8H, m, DMT), 5.44 (1H, dd, J = 2.3, 8.2 Hz, H5), 4.39 (1H, bs, H4′), 4.32−4.28 (1H, m, H5′), 3.78 (3H, s, OMe), 3.78 (3H, s, OMe), 3.73 (3H, s, OMe), 3.72 (3H, s, OMe), 3.61 (1H, dd, J = 6.0, 10.4 Hz, H6′), 3.55 (1H, d, J = 9.5 Hz, H1′), 3.51 (1H, dd, J = 4.4, 10.4 Hz, H6′), 3.42 (1H, d, J = 9.5 Hz, H1′), 2.81 (1H, bs, OH), 2.98 (1H, d, J = 15.0 Hz, H3′), 2.32 (1H, dd, J = 4.6, 15.2 Hz, H3′); ^{13}C NMR ($CDCl_3$) δ 163.96 (C4), 158.54, 158.52, 158.34 (DMT), 149.73 (C2), 144.55, 144.34 (DMT), 141.63 (C6), 135.67, 135.53, 135.42, 129.94, 129.87, 129.81, 128.99, 127.99, 127.93, 127.85, 127.67, 126.91, 126.69, 113.15, 113.00, 112.99 (DMT), 99.92 (C5), 98.48 (C2′), 86.61, 86.06 (Ar_2PhC), 84.37 (C5′), 71.23 (C4′), 65.25 (C1′), 62.16 (C6′), 55.12, 55.04 (OMe), 41.37 (C3′). Anal. Calcd for $C_{52}H_{50}N_2O_{10}$: C, 72.4; H, 5.8; N, 3.3. Found: C, 72.1; H, 5.8; N, 3.6.

Alternative Preparation of 8. Compound **9** (4.313 g, 5.00 mmol) was dissolved in anhydrous pyridine (30 mL) and cooled to 0 °C, methanesulfonyl chloride (1.5 mL, 19.3 mmol) was added, and the mixture was stirred at room temperature for 3 h. Saturated aqueous $NaHCO_3$ (100 mL) and H_2O (100 mL) were added, and the suspension was extracted with EtOAc (4 × 100 mL). The combined organic phase was washed with H_2O (2 × 80 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (3 × 100 mL). The resulting crude residue **10** was dissolved in anhydrous DMF (60 mL), NaN_3 (3.011 g, 46.3 mmol) was added, and the suspension was stirred at 70 °C for 2 days. Saturated aqueous $NaHCO_3$ (100 mL) and H_2O (100 mL) were added, and the mixture was extracted with EtOAc (4 × 100 mL). The combined organic phase was washed with H_2O (2 × 80 mL), evaporated to dryness under reduced pressure and coevaporated with CH_2Cl_2/*n*-hexane (1:4 (v/v), 2 × 100 mL). The residue was purified by silica gel column chromatography (13.3 × 5.5 cm) eluting with a gradient of 0.5:0−5:99.5−94.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) to give compound **8** (4.297 g, 97%) as a white foam after coevaporation with acetonitrile (3 × 50 mL). Physical data were identical with those obtained earlier.

1-[4′-Azido-3′-deoxy-β-D-psicofuranosyl]uracil (11). Azide **8** (4.247 g, 4.78 mmol) was dissolved in CH_2Cl_2 (10 mL) and 80% aqueous AcOH (50 mL), and the solution was stirred for 4 h, evaporated to dryness under reduced pressure, and coevaporated with absolute EtOH (100 mL). MeOH (100 mL) and silica gel (50 mL) were added, and the suspension was evaporated under reduced pressure and coevaporated with CH_2Cl_2 (100 mL). The residue was purified by silica gel column chromatography (12 × 5.5 cm) eluting with a gradient of 5−10% MeOH in CH_2Cl_2 (v/v) to give nucleoside **1** (1.091 g, 81%) as a white foam: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.20; FAB-MS m/z 284 $[M + H]^+$; ^1H NMR (CD_3OD) δ 8.06 (1H, d, J = 8.2 Hz, H6), 5.60 (1H, dd, J = 1.5, 8.0 Hz, H5), 4.19 (1H, ddd, J = 5.2, 5.7, 7.2 Hz, H4′), 4.10 (1H, q, J = 4.6 Hz, H5′), 3.94 (1H, d, J = 11.9 Hz, H1′), 3.77 (1H, d, J = 11.9 Hz, H1′), 3.72 (1H, dd, J = 4.0, 12.1 Hz, H6′), 3.63 (1H, dd, J = 4.7, 12.1 Hz, H6′), 3.05 (1H, dd, J = 7.1, 14.7 Hz, H3′), 2.41 (1H, dd, J = 5.8, 14.7 Hz, H3′); ^{13}C NMR (CD_3OD) δ 167.06 (C4), 152.01 (C2), 144.06 (C6), 100.58, 100.41 (C2′, C5), 87.69 (C5′), 65.69 (C1′), 62.14 (C6′), 61.66 (C4′), 40.28 (C3′).

1-[(2,1′-Anhydro)-4′-azido-3′-deoxy-6′-*O*-(*p*-toluenesulfonyl)-β-D-psicofuranosyl]uracil (12). Nucleoside **11** (117 mg, 0.41 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) and anhydrous pyridine (2 mL), TsCl (510 mg, 2.68 mmol) was added, and the mixture was stirred at room temperature for 45 h. Saturated aqueous $NaHCO_3$ (10 mL) and H_2O (10 mL) were added, and the suspension was extracted with EtOAc (5 × 20 mL). The combined organic phase was washed with H_2O (2 × 20 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (2 × 20 mL). The residue was purified by silica gel column chromatography (12.5 × 2.8 cm) eluting with a gradient of 2−4% MeOH in CH_2Cl_2 (v/v) to give anhydronucleoside **12** (109 mg, 65%) as a light yellow foam: R_f (20% MeOH in CH_2Cl_2 (v/v)) 0.72; FAB-MS m/z 420 $[M + H]^+$; ^1H NMR ($CDCl_3$) δ 7.77 (2H, d, J = 8.4 Hz, Ts), 7.42 (1H, d, J = 7.7 Hz, H6), 7.39 (2H, d, J = 7.9 Hz, Ts), 6.03 (1H, d, J = 7.5 Hz, H5), 4.64 (1H, d, J = 10.4 Hz, H1′), 4.60 (1H, d, J = 10.4 Hz, H1′), 4.51 (1H, dt, J = 3.2, 6.4 Hz, H4′), 4.25 (1H, dd, J = 2.3, 11.1 Hz, H6′), 4.20 (1H, q, J = 2.6 Hz, H5′), 4.16 (1H, dd, J = 2.8, 11.1 Hz, H6′), 2.97 (1H, dd, J = 6.6, 14.5 Hz, H3′), 2.47 (1H, dd, J = 3.5, 14.5 Hz, H3′), 2.47 (3H, s, $ArCH_3$); ^{13}C NMR ($CDCl_3$) δ 171.41 (C4), 159.85 (C2), 145.96, 132.60 (Ts), 131.59 (C6), 130.18, 127.73 (Ts), 110.70 (C5), 97.70 (C2′), 82.35 (C5′), 77.40 (C1′), 68.38 (C6′), 61.30 (C4′), 39.87 (C3′), 21.61 ($ArCH_3$).

1-[4′-Azido-3′-deoxy-1′,6′-di-*O*-methanesulfonyl-β-D-psicofuranosyl]uracil (13). Nucleoside **11** (931 mg, 3.29 mmol) was dissolved in anhydrous anhydrous pyridine (20 mL) at 0 °C, MsCl (1.25 mL, 16.1 mmol) was added, and stirring was continued at 0 °C for 1 h 30 min. EtOAc (200 mL) was added, and the mixture was washed with H_2O (2 × 40 mL). The combined aqueous phase was extracted with CH_2Cl_2 (3 × 40 mL), and the combined organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (3 × 60 mL). The residue was purified by silica gel column chromatography (7.5 × 5.5 cm) eluting with a gradient of 2−3% MeOH in CH_2Cl_2 (v/v) to give nucleoside **13** (1329 mg, 92%) as a white foam: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.35; IR (KBr) ν 2116 cm^{-1} (strong); FAB-MS m/z 440 $[M + H]^+$; ^1H NMR (acetone-d_6) δ 10.07 (1H, bs, NH), 7.91 (1H, d, J = 8.4 Hz, H6), 5.60 (1H, d, J = 8.4 Hz, H5), 4.78 (1H, d, J = 11.0 Hz, H1′), 4.59−4.48 (5H, m, H1′, H4′, H5′, H6′), 3.27 (1H, dd, J = 6.5, 14.9 Hz, H3′), 3.19 (3H, s, Me), 3.14 (3H, s, Me), 2.65 (1H, dd, J = 4.9, 15.0 Hz, H3′); ^{13}C NMR (acetone-d_6) δ 163.94 (C4), 151.24 (C2), 141.26 (C6), 101.69 (C5), 97.26 (C2′), 84.61 (C5′), 71.10 (C1′), 68.56 (C6′), 61.25 (C4′), 41.03 (C3′), 37.69, 37.55 (Me).

(1*S*,3*R*,4*S*)-3-(Methanesulfonyloxymethyl)-1-(uracil-1-yl)-5-aza-2-oxabicyclo[2.2.1]heptane (14). Compound **13** (1329 mg, 3.02 mmol) was dissolved in CH_2Cl_2/MeOH (5 mL, 9:1 (v/v)), MeOH (25 mL) and Pd/C (10% w/w, 113 mg) suspended in MeOH (3 mL) were added ,and the suspension was evacuated with H_2 several times. After stirring under an H_2 atmosphere for 25 h, the suspension was evaporated on silica gel, and the residue was purified by a short silica gel column (4.2 × 2.8 cm) eluting with a gradient of 0.5:10−20:89.5−79.5 Et_3N/MeOH/CH_2Cl_2 (v/v/v) to give an intermediate as a white foam after coevaporation with acetonitrile (100 mL). This intermediate was dissolved in anhydrous DMF (50 mL) and stirred at 60 °C for 19 h. After cooling, the solution was evaporated to dryness under reduced pressure, coevaporated with acetonitrile (3 × 30 mL), dissolved in MeOH/CH_2Cl_2 (200 mL, 1:1 (v/v)), and evaporated on silica gel. The residue was purified by silica gel column chromatography (14.3 × 2.8 cm) eluting with a gradient of 0.5:10−20:89.5−79.5 Et_3N/MeOH/CH_2Cl_2 (v/v/v) to give bicyclic amine **14** (724 mg, 75%) as a light yellowish foam after coevaporation with acetonitrile (3 × 20 mL): R_f (20% MeOH in CH_2Cl_2 (v/v)) 0.40; FAB-MS *m*/*z* 318 $[M + H]^+$; ^{1}H NMR (DMSO-d_6) δ 11.48 (1H, bs, NH), 9.35 (1H, bs, NH), 7.79 (1H, d, J = 8.2 Hz, H6), 5.69 (1H, d, J = 8.2 Hz, H5), 4.46 (2H, m, H4′, H5′), 4.30 (1H, dd, J = 4.9, 11.4 Hz, H6′), 4.23 (1H, dd, J = 6.2, 11.4 Hz, H6′), 3.99 (1H, dd, J = 1.3, 11.7 Hz, H1$_{endo}$′), 3.66 (1H, d, J = 11.5 Hz, H1$_{exo}$′), 3.21 (3H, s, Me), 2.84 (1H, d, J = 11.7 Hz, H3$_\beta$′), 2.33 (1H, m, H3$_\alpha$′); ^{13}C NMR (DMSO-d_6) δ 163.11 (C4), 150.08 (C2), 143.14 (C6), 101.83 (C5), 97.02 (C2′), 82.20 (C5′), 69.66 (C6′), 56.32 (C4′), 52.96 (C1′), 36.88 (Ms), 36.75 (C3′).

(1*S*,3*R*,4*S*)-3-(Methanesulfonyloxymethyl)-5-*N*-(4-monomethoxytrityl)-1-(uracil-1-yl)-5-aza-2-oxabicyclo[2.2.1]heptane (15). Amine **14** (697 mg, 2.20 mmol) was coevaporated with anhydrous pyridine (40 mL), dissolved in anhydrous pyridine (60 mL), and evaporated to half volume. MMTCl (2.065 g, 6.69 mmol) was added, and the mixture was stirred for 21 h. Saturated aqueous $NaHCO_3$ (10 mL) was added, the suspension was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (3 × 25 mL), and the residue was suspended in MeOH (50 mL) and evaporated on silica gel. The residue was purified by silica gel column chromatography (13.3 × 2.8 cm) eluting with a gradient of 0.5:0.5−1:99−98.5 Et_3N/MeOH/CH_2Cl_2 (v/v/v) to yield nucleoside **15** (1032 mg, 80%) as a white foam after coevaporation with acetonitrile (3 × 20 mL): R_f (5% MeOH in CH_2Cl_2 (v/v)) 0.21; FAB-MS *m*/*z* 590 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.9 (1H, bs, NH), 7.52 (4H, m, MMT), 7.43 (2H, d, J = 9.0 Hz, MMT), 7.38 (1H, d, J = 8.2 Hz, H6), 7.31−7.18 (6H, m, MMT), 6.84 (2H, d, J = 9.2 Hz, MMT), 5.61 (1H, d, J = 8.2 Hz, H5), 4.72 (1H, m, H5′), 4.14 (1H, dd, J = 5.5, 11.0 Hz, H6′), 3.97 (1H, dd, J = 6.8, 11.0 Hz, H6′), 3.77 (3H, s, OMe), 3.70 (1H, s, H4′), 3.60 (1H, d, J = 9.7 Hz, H1$_{endo}$′), 3.23 (1H, d, J = 9.5 Hz, H1$_{exo}$′), 3.00 (3H, s, Ms), 1.71 (1H, d, J = 10.6 Hz, H3$_\beta$′), 0.78 (1H, dd, J = 1.8, 10.4 Hz, H3$_\alpha$′); ^{13}C NMR ($CDCl_3$) δ 162.81 (C4), 158.07 (MMT),148.94 (C2), 143.51 (MMT), 141.39 (C6), 135.06, 130.11, 128.77, 127.95, 126.57, 126.54, 113.31 (MMT), 102.05 (C5), 96.22 (C2′), 82.92 (C5′), 75.95 (ArPh$_2$*C*), 67.92 (C6′), 59.21 (C4′), 56.41 (C1′), 55.11 (OMe), 37.52 (Ms), 35.06 (C3′).

(1*S*,3*R*,4*S*)-3-(Hydroxymethyl)-5-*N*-(4-monomethoxytrityl)-1-(uracil-1-yl)-5-aza-2-oxabicyclo[2.2.1]heptane (17). Compound **15** (180 mg, 0.305 mmol) was dissolved in anhydrous DMF (30 mL), NaOBz (445 mg, 3.09 mmol) was added, and the suspension was stirred at 100 °C under an N_2 atmosphere for 22 h. After cooling, EtOAc (100 mL) was added, and the mixture was washed with H_2O (3 × 25 mL). The combined aqueous phase was extracted with EtOAc (3 × 25 mL), and the combined organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (50 mL), *n*-hexane (2 × 100 mL), and *n*-hexane/acetonitrile (2 × 100 mL 4:1 (v/v)). Crude **16** (see below) was dissolved in MeOH (20 mL), NaOMe (122 mg, 2.26 mmol) was added, and the suspension was stirred for 13 h. The mixture was evaporated to dryness under reduced pressure, suspended in CH_2Cl_2 (20 mL), and evaporated on silica gel. The residue was purified by silica gel column chromatography (8.3 × 2.8 cm) eluting with a gradient of 0.5:10−20:89.5−79.5 Et_3N/acetone/CH_2Cl_2 (v/v/v) followed by 0.5:5:94.5 Et_3N/MeOH/CH_2Cl_2 to give nucleoside **17** (121 mg, 78%) as a white foam after coevaporation with acetonitrile (3 × 40 mL), CH_2Cl_2/*n*-hexane (3 × 15 mL, 1:4 (v/v)), and acetonitrile (20 mL): R_f (8% MeOH in CH_2Cl_2 (v/v)) 0.48; FAB-MS *m*/*z* 512 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.53 (4H, d, J = 7.7 Hz, MMT), 7.44 (2H, d, J = 8.6 Hz, MMT), 7.35 (1H, d, J = 8.2 Hz, H6), 7.30−7.17 (6H, m, MMT), 6.82 (2H, d, J = 9.0 Hz, MMT), 5.58 (1H, d, J = 8.2 Hz, H5), 4.60 (1H, t, J = 5.5 Hz, H5′), 3.76 (3H, s, OMe), 3.64 (1H, d, J = 9.7 Hz, H1$_{endo}$′), 3.62 (1H, s, H4′), 3.56 (1H, dd, J = 5.2, 12.0 Hz, H6′), 3.52 (1H, dd, J = 5.7, 11.9 Hz, H6′), 3.16 (1H, d, J = 9.5 Hz, H1$_{exo}$′), 1.85 (1H, d, J = 10.3 Hz, H3$_\beta$′), 0.57 (1H, dd, J = 1.7, 10.3 Hz, H3$_\alpha$′); ^{13}C NMR ($CDCl_3$) δ 162.87 (C4), 157.96 (MMT),149.14 (C2), 143.70 (MMT), 141.71 (C6), 130.13, 128.81, 127.86, 126.42, 126.41, 113.21 (MMT), 101.81 (C5), 96.05 (C2′), 86.68 (C5′), 75.95 (ArPh$_2$*C*), 63.36 (C6′), 59.38 (C4′), 56.62 (C1′), 55.08 (OMe), 35.40 (C3′).

(1*S*,3*R*,4*S*)-3-(Benzoyloxymethyl)-5-*N*-(4-monomethoxytrityl)-1-(uracil-1-yl)-5-aza-2-oxabicyclo[2.2.1]heptane (16). Physical data of the intermediary benzoate **16** purified by silica gel column chromatography eluting with a gradient of 0.5:0−10:99.5−89.5 pyridine/acetone/CH_2Cl_2 (v/v/v): R_f (1:4 acetone/CH_2Cl_2 (v/v)) 0.67; FAB-MS *m*/*z* 616 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.55 (1H, bs, NH), 8.00 (2H, dd, J = = 1.4, 8.4 Hz, Bz), 7.62−7.53 (4H, m, MMT), 7.49 (1H, d, J = 8.2 Hz, H6), 7.47−7.43 (4H, m, Bz, MMT), 7.29−7.17 (6H, m, MMT), 6.80 (2H, d, J = 9.2 Hz, MMT), 5.62 (1H, d, J = 8.2 Hz, H5), 4.86 (1H, dd, J = 4.9, 8.2 Hz, H5′), 4.33 (1H, dd, J = 4.9, 11.5 Hz, H6′), 4.03 (1H, dd, J = 8.4, 11.5 Hz, H6′), 3.76 (1H, bs, H4′), 3.74 (3H, s, OMe), 3.58 (1H, d, J = 9.7 Hz, H1$_{endo}$′), 3.29 (1H, d, J = 9.7 Hz, H1$_{exo}$′), 1.64 (1H, d, J = 10.4 Hz, H3$_\beta$′), 0.89 (1H, dd, J = 2.1, 10.4 Hz, H3$_\alpha$′); ^{13}C NMR ($CDCl_3$) δ 165.79 (Ph*C*O), 162.72 (C4), 157.97 (MMT), 148.78 (C2), 143.64 (MMT), 141.50 (C6), 135.44 (MMT), 133.17 (Bz), 130.09, 129.61, 129.39, 129.07, 128.84, 128.29, 127.87, 127.70, 126.41 (Bz, MMT), 113.23, 113.05 (MMT), 101.81 (C5), 96.03 (C2′), 83.50 (C5′), 75.98 (ArPh$_2$*C*), 63.98 (C6′), 59.60 (C4′), 56.41 (C1′), 55.05 (OMe), 34.88 (C3′).

(1*S*,3*R*,4*S*)-3-(2-Cyanoethoxy(diisopropylamino)phosphinoxymethyl)-5-*N*-(4-monomethoxytrityl)-1-(uracil-1-yl)-5-aza-2-oxabicyclo[2.2.1]heptane (18). Compound **17** (234 mg, 0.458 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL), *N*,*N*-diisopropylethylamine (1.0 mL) followed by 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.50 mL, 2.2 mmol) was added, and the mixture was stirred at room temperature for 20 h. H_2O (5 mL) and EtOAc (200 mL) were added, and the mixture was washed with saturated aqueous $NaHCO_3$ (40 mL) and H_2O (2 × 40 mL). The combined organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (2 × 100 mL), and the residue was purified by silica gel column chromatography (17.5 × 2.8 cm) eluting with 0.5:50:49.5 Et_3N/EtOAc/*n*-hexane (v/v/v) to give phosphoramidite **18** (237 mg, 73%) as a white foam after coevaporation with acetonitrile (4 × 5 mL): R_f (8% MeOH in CH_2Cl_2 (v/v)) 0.49; FAB-MS *m*/*z* 712 $[M + H]^+$; ^{31}P NMR ($CDCl_3$) δ 149.67, 149.41. Anal. Calcd for $C_{39}H_{46}N_5O_6P$: C, 65.8; H, 6.5; N, 9.8. Found: C, 64.8; H, 6.6; N, 10.0.

Oligonucleotide Synthesis. All oligomers were synthesized on a DNA synthesizer using the phosphoramidite approach.[25] The stepwise coupling yields were determined spectrophotometrically at 498 nm (quantifying the released 4,4′-dimethoxytrityl group). Standard conditions were used for commercial amidites except for a 10-min coupling time for the "inverse" 3′-*O*-DMT-5′-*O*-(2-cyanoethoxy(diisopropylamino)-phosphino)-2′-deoxyadenosine, -2′-deoxycytidine, and -2′-deoxyguanosine (purchased from ChemGenes). The modified amidite **18** was "hand-coupled" [premixing a ∼0.05 M solution of amidite in anhydrous acetonitrile (0.2 mL, 10 μmol) and a ∼0.5 M solution of tetrazole in anhydrous acetonitrile (0.3 mL, 150 μmol) in a syringe and via an adaptor slowly flushing this mixture through a synthesis column for 12 min]. Combined

(25) Caruthers, M. H. *Acc. Chem. Res.* **1991**, *24*, 278−284.

coupling yields for attachment of the amidite **18** and the concomitant coupling step of an inverse amidite onto the amino group were ~90%, while coupling yields for the inverse and the unmodified amidites were ~99% and >99%, respectively. Cleavage from the solid support and removal of protecting groups was accomplished using concentrated aqueous ammonia (55 °C for 16 h). A BioGene X Unisupport was used for the modified oligonucleotide, which was cleaved off by treatment with 2% LiCl during deblocking. Unmodified 9-mers were purified by desalting using a Pharmacia NAP-10 column. The modified 9-mer was purified by preparative anion-exchange FPLC (Pharmacia Resource Q 1-mL column) eluting with a gradient of 0−50% of a 10 mM NaOH/1.5 M NaCl buffer in 100−50% 10 mM NaOH followed by desalting on a Pharmacia NAP-25 column. Capillary gel electrophoresis of all synthesized sequences showed >90% purity. The composition of the modified 9-mer was verified by MALDI-MS analysis: m/z $[M - H]^-$ 2746.7 (found mass); 2743.5 (calcd mass).

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Supporting Information Available: Copies of ^{13}C NMR spectra for **4**−**9** and **11**−**17**, a copy of the ^{31}P NMR spectrum for **18**, and a copy of the transcript from the capillary gel electrophoretic analysis of the modified 9-mer oligonucleotide (entry 3, Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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