Novel Bicyclic Nucleoside Analogue (1*S*,5*S*,6*S*)-6-Hydroxy-5-hydroxymethyl-1-(uracil-1-yl)-3,8-dioxabicyclo[3.2.1]octane: Synthesis and Incorporation into Oligodeoxynucleotides

Lisbet Kværnø[†] and Jesper Wengel*[,‡]

Center for Synthetic Bioorganic Chemistry, Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark, and Department of Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

jwe@chem.sdu.dk

Received April 4, 2001

The novel bicyclic nucleoside (1*S*,5*S*,6*S*)-6-hydroxy-5-hydroxymethyl-1-(uracil-1-yl)-3,8-dioxabicyclo-[3.2.1]octane [2′-deoxy-1′-*C*,4′-*C*-(2-oxapropano)uridine] (**15**), expected to be restricted into an O4′-endo furanose conformation, was synthesized from the known 1-(3′-deoxy-*β*-D-psicofuranosyl)uracil **5**. The phosphoramidite derivative of **15** was successfully incorporated into oligodeoxynucleotides using standard methods, and thermal denaturation studies showed moderate decreases in duplex stabilities of −2.1 and −1.5 °C per modification toward complementary DNA and RNA, respectively.

Introduction

The promise of the efficient inhibition of gene expression as described by the antisense approach[1] has led to the synthesis and investigation of multiple conformationally restricted nucleoside analogues. Many of the differences in hybridization properties between chemically modified oligonucleotides can be described by one single parameter, the pseudorotation angle, which defines the furanose conformation of each single nucleotide monomer.[2] The C3′-endo (or in general N-type) conformation leading to A-type duplexes as seen for natural RNA generally effects the highest duplex stabilities.[3−5] Examples of such nucleotide monomers are LNA **1** (locked nucleic acid, Figure 1), which displays unprecedented binding affinity toward complementary DNA and RNA,[6−9] and the C2′-methylene extended derivative **2** synthesized with thymine and cytosine bases,[10] which also displays highly stable duplexes with complementary RNA.[11] X-ray studies revealed both nucleosides to be restricted into C3′-endo conformations,[10,12] while NMR studies of LNA single strands hybridized to complementary DNA and RNA verified the expected A-type duplexes.[13,14]

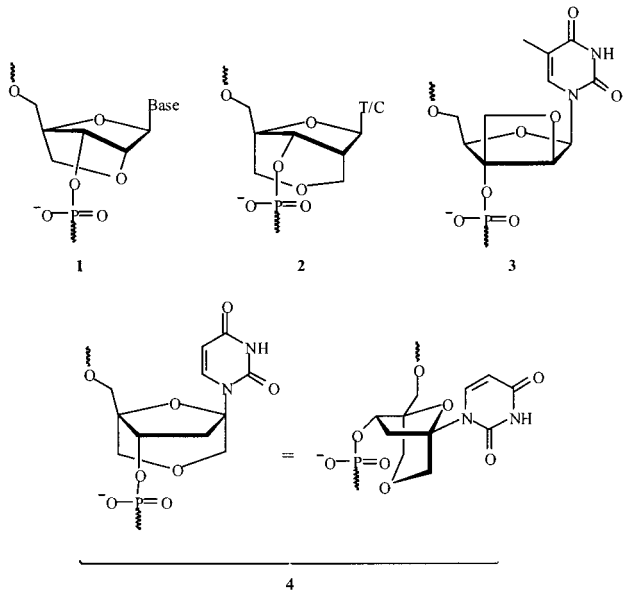


Figure 1. Structures of four bicyclic nucleotide monomers. **1** (LNA) and **2** are restricted to C3′-endo (N-type) furanose conformations, while **3** and the novel **4** are restricted to O4′-endo (E-type) furanose conformations.

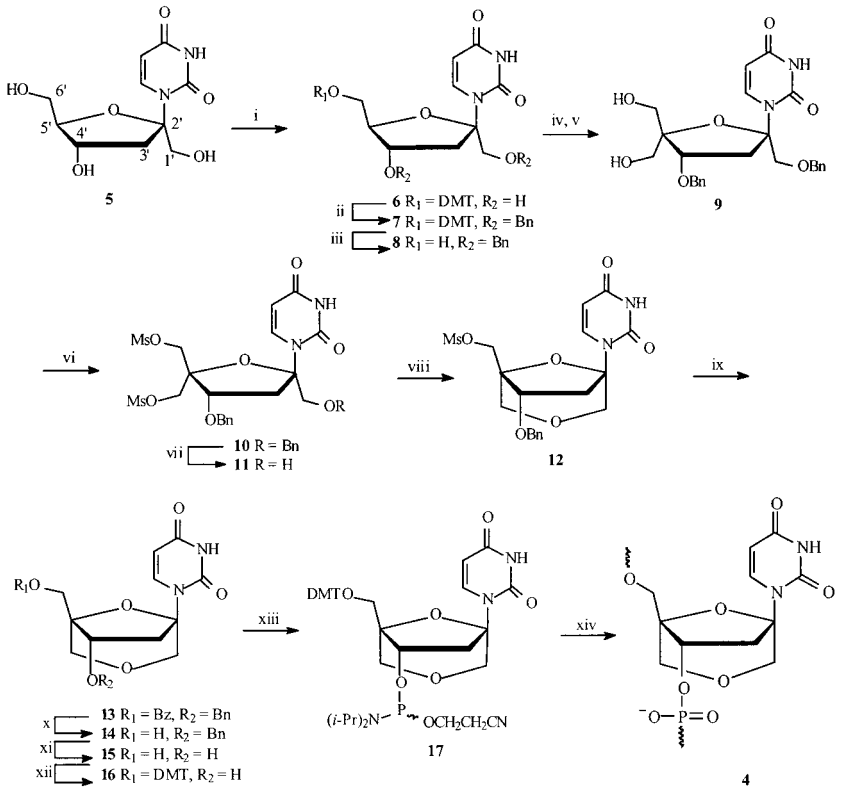
The capability to activate RNase H, the enzyme which cleaves the RNA strand in RNA/DNA heteroduplexes, thus allowing a single antisense oligonucleotide to target multiple RNA strands, is another desired feature for antisense oligonucleotides. In general, oligonucleotides preorganized into a C3′-endo conformation do *not* activate RNase H.[4] So far, only two types of fully modified oligonucleotide analogues with an altered carbohydrate part display this ability, namely arabino nucleic acids[15,16] and cyclohexene nucleic acids.[17] The 2′-F-arabino nucleic

[†] University of Copenhagen.
[‡] University of Southern Denmark.

(1) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543−584.
(2) Altona, C.; Sunderalingam, M. *J. Am. Chem. Soc.* **1972**, *94*, 8205−8212.
(3) Freier, S. M.; Altmann, K. H. *Nucleic Acids Res.* **1997**, *25*, 4429−4443.
(4) Cook, P. D. *Nucleosides Nucleotides* **1999**, *18*, 1141−1162.
(5) Herdewijn, P. *Biochim. Biophys. Acta* **1999**, *1489*, 167−179.
(6) Singh, S. K.; Nielsen, P.; Koshkin, A. A.; Wengel, J. *Chem. Commun.* **1998**, 455−456.
(7) Koshkin, A. A.; Singh, S. K.; Nielsen, P.; Rajwanshi, V. K.; Kumar, R.; Meldgaard, M.; Olsen, C. E.; Wengel, J. *Tetrahedron* **1998**, *54*, 3607−3630.
(8) Koshkin, A. A.; Nielsen, P.; Meldgaard, M.; Rajwanshi, V. K.; Singh, S. K.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 13252−13253.
(9) Obika, S.; Nanbu, D.; Hari, Y.; Andoh, J.; Morio, K.; Doi, T.; Imanishi, T. *Tetrahedron Lett.* **1998**, *39*, 5401−5404.
(10) Wang, G. Y.; Girardet, J. L.; Gunic, E. *Tetrahedron* **1999**, *55*, 7707−7724.
(11) Wang, G. Y.; Gunic, E.; Girardet, J. L.; Stoisavljevic, V. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1147−1150.
(12) Obika, S.; Nanbu, D.; Hari, Y.; Morio, K.; In, Y.; Ishida, T.; Imanishi, T. *Tetrahedron Lett.* **1997**, *38*, 8735−8738.
(13) Petersen, M.; Nielsen, C. B.; Nielsen, K. E.; Jensen, G. A.; Bondensgaard, K.; Singh, S. K.; Rajwanshi, V. K.; Koshkin, A. A.; Dahl, B. M.; Wengel, J.; Jacobsen, J. P. *J. Mol. Recognit.* **2000**, *13*, 44−53.
(14) Bondensgaard, K.; Petersen, M.; Singh, S. K.; Rajwanshi, V. K.; Kumar, R.; Wengel, J.; Jacobsen, J. P. *Chem.—Eur. J.* **2000**, *6*, 2687−2695.

10.1021/jo015664l CCC: $20.00
Published on Web 07/19/2001

Scheme 1[a]



[a] Reagents and conditions: (i) ref 23, 63%; (ii) BnBr, NaH, THF, 85%; (iii) 80% aq AcOH, 94%; (iv) Dess−Martin periodinane, CH_2Cl_2; (v) H_2CO (37%), 1 M NaOH (aq), 1,4-dioxane, 78% (2 steps); (vi) MsCl, pyridine; (vii) H_2, 20% $Pd(OH)_2$/C, EtOH, 55% from **9**; (viii) 1 M NaOH (aq), 1,4-dioxane, 64%; (ix) NaOBz, DMF; (x) NaOMe, MeOH, 90% (2 steps); (xi) BCl_3, CH_2Cl_2, 84%; (xii) DMTCl, pyridine, 67%; (xiii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 26%; (xiv) DNA synthesizer. DMT = 4,4′-dimethoxytrityl.

acid, which also increases the binding affinity toward complementary RNA, was shown by X-ray crystallography to adopt an O4′-endo furanose conformation,[18,19] which is a high-energy conformation and therefore is very unusual for natural nucleic acids.[2] Similar hybridization properties toward complementary RNA, as well as higher duplex stabilities with complementary DNA, were obtained for the bicyclic monomer **3,** which was shown by molecular modeling and NMR studies likewise to be restricted into an O4′-endo conformation.[20]

To further investigate this unusual O4′-endo conformation, we decided to synthesize the novel bicyclic nucleoside **4** in which the furanose ring is also expected to be restricted into an O4′-endo conformation. Although the additional ring system is sterically rather large, the increased duplex stabilities for the similar bicycle **2** when incorporated into oligonucleotides suggest no serious sterical restraints upon duplex formation for such a bicyclic system.

Results and Discussion

The known 1-(3′-deoxy-β-D-psicofuranosyl)uracil **5**[21,22] is preferentially 4,4′-dimethoxytritylated in the 6′ position (refer to Scheme 1, structure **5**, for numbering used throughout the discussion). Using methods similar (DMTCl in pyridine; 4,4'-dimethoxytrityl) to those previously described,[23] but with the regeneration of starting nucleoside **5** from undesired DMT-protected isomers by treatment with 80% AcOH and repeated tritylation, a total 63% yield of nucleoside **6** was obtained (data not shown). The remaining hydroxy groups were selectively benzylated using BnBr and NaH in THF to give **7** in a reaction where intermediate workup before addition of extra NaH and BnBr to complete the dibenzylation appeared to minimize the formation of the tribenzylated byproduct. The DMT group was hydrolyzed with 80% AcOH, affording **8** in 94% yield, whereupon oxidation to the intermediary aldehyde was accomplished with Dess−Martin periodinane[24] in CH_2Cl_2. A tandem aldol condensation and Cannizzaro reaction using aqueous formaldehyde and 1 M NaOH in 1,4-dioxane furnished the diol **9** in 78% combined yield. Mesylation of the hydroxy groups (MsCl in pyridine) proceeded smoothly, and at this stage, a selective debenzylation of the primary hydroxy

(15) Damha, M. J.; Wilds, C. J.; Noronha, A.; Brukner, I.; Borkow, G.; Arion, D.; Parniak, M. A. *J. Am. Chem. Soc.* **1998**, *120*, 12976−12977.
(16) Noronha, A. M.; Wilds, C. J.; Lok, C. N.; Viazovkina, K.; Arion, D.; Parniak, M. A.; Damha, M. J. *Biochemistry* **2000**, *39*, 7050−7062.
(17) Wang, J.; Verbeure, B.; Luyten, I.; Lescrinier, E.; Froeyen, M.; Hendrix, C.; Rosemeyer, H.; Seela, F.; Van Aerschot, A.; Herdewijn, P. *J. Am. Chem. Soc.* **2000**, *122*, 8595−8602.
(18) Berger, I.; Tereshko, V.; Ikeda, H.; Marquez, V. E.; Egli, M. *Nucleic Acids Res.* **1998**, *26*, 2473−2480.
(19) Minasov, G.; Teplova, M.; Nielsen, P.; Wengel, J.; Egli, M. *Biochemistry* **2000**, *39*, 3525−3532.
(20) Christensen, N. K.; Petersen, M.; Nielsen, P.; Jacobsen, J. P.; Olsen, C. E.; Wengel, J. *J. Am. Chem. Soc.* **1998**, *120*, 5458−5463.
(21) Holý, A. *Nucleic Acids Res.* **1974**, *1*, 289−298.
(22) Ono, A.; Dan, A.; Matsuda, A. *Bioconjugate Chem.* **1993**, *4*, 499−508.
(23) Kværnø, L.; Nielsen, C.; Wightman, R. H. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2903−2906.
(24) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277−7287.

group was feasible by catalytic hydrogenation using 20% $Pd(OH)_2/C$ as the catalyst, which afforded 55% combined yield of **11** and only 11% combined yield of the isomer resulting from the removal of both benzyl groups (referred to as **11B** in the Experimental Section). It might be worthwhile to note that an alternative tosylation of **9** (TsCl in pyridine or TsCl and DMAP in CH_2Cl_2) proceeded in only 51% and 46% yields, respectively, and that the selective debenzylation was not reproducible for the tosylated isomer of **10**. The cyclization of **11** was performed by treatment with 1 M NaOH (aq) in dioxane at 90 °C overnight to give 64% yield of **12**, while no trace of simultaneous hydrolysis of the mesyl group was detectable. Therefore, a two-step sequence for the removal of the mesyl group was applied, performing first a nucleophilic substitution with NaOBz in DMF at 100 °C to give the intermediate benzoate **13**, followed by methanolysis using NaOMe in MeOH, yielding **14** in 90% yield (from **12**). Surprisingly, hydrogenolysis of the benzyl group using 20% $Pd(OH)_2/C$ in EtOH under an atmosphere of hydrogen yielded an inseparable 1:1 mixture of **15** together with the debenzylated product where also the double bond of the uracil base was hydrogenated. Alternatively, DMT-protection of **14** (R_1 = DMT) followed by hydrogenation with ammonium formate as the hydrogen donor and 10% Pd/C as the catalyst in refluxing MeOH afforded quantitative reduction of the nucleobase with no significant loss of the DMT protecting group. Thus, debenzylation by Lewis acids was applied instead, which yielded the desired nucleoside **15** in 84% yield after treatment with BCl_3 in CH_2Cl_2. Selective DMT protection of the primary hydroxy group using DMTCl in pyridine yielded **16** in 67% yield, and last, 3′-*O*-phosphitylation using 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochlorodite and *N*,*N*-diisopropylethylamine in CH_2Cl_2 furnished the phosphoramidite **17**. The yield of the phosphitylation reaction was only 26%, which most likely can be explained, at least in part, by the difficult removal of some phosphorus-containing impurities requiring severel chromatographic purifications.

The bicyclic structure obtained after the cyclization to **12** was verified by HMBC (heteronuclear multiple bond correlation) NMR showing two- and three-bond C−H correlations, because the pairs of atoms H6″/C1′ and H1′/C6″ appeared to be maximally three bonds apart. An energy-minimized structure of **15** obtained by molecular mechanics using MacroModel v.7.0[25] [pseudosystematical Monte Carlo conformational search[26] (1000 steps, limit 50 kJ/mol) with water as solvent, MMFF force field[27]] supported the assumption of an O4′-endo furanose conformation, while the six-membered ring adopted a chairlike conformation with one oxygen pointing toward 3′-OH (sketched in Figure 1). Thus, out of the 79 discrete conformations found, the 73 lowest-energy conformers all displayed an O4′-endo furanose conformation and a chairlike conformation of the 1,4-dioxane ring (see Supporting Information for further details).

Conformational analysis by NOE difference spectra recorded for the bicyclic derivatives **14** and **15** was not conclusive with respect to a certain furanose conformation, because H3′ and H4′ appeared only to be mutually close, thus not contradicting the result from the modeling. Values of $^3J_{3a',4'}$ and $^3J_{3b',4'}$ for compounds **12**−**16** in the ranges 3.8−4.4 and 10.3−11.2 Hz, respectively, preclude an extreme *S*-type (C2′-endo) conformation, though.[28]

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)	29.4		26.6	
2	5′-d(GTGA**U**ATGC)	27.9		26.4	
3	5′-d(GTGA**4**ATGC)	25.8	−2.1	25.8	−0.8
4	5′-d(G**U**GA**U**A**U**GC)	27.3		25.6	
5	5′-d(G**4**GA**4**A**4**GC)	18.7	−2.9	21.0	−1.5

[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; d**U** = 2′-deoxyuridine monomer; dT = thymidine monomer; **4**, see Figure 1. All abbreviated as d(sequence).

Phosphoramidite **17** was incorporated once and three times as monomer **4** (Figure 1) into the mixed 9-mer oligodeoxynucleotide sequence depicted in Table 1 (entries 3 and 5, respectively). Standard conditions were used, except for a 10 min coupling time for the amidite **17** which afforded a coupling yield of ∼98% compared with >99% yield for the unmodified amidites (refer to Experimental Section for further details). The oligonucleotides were purified by ethanol precipitation, and capillary gel electrophoresis showed >90% purity of the products, whose compositions were verified from MALDI-MS analysis.

As depicted in Table 1, the melting temperatures (T_m values) and the changes in T_m value per modification, compared with those of the unmodified reference containing 2′-deoxyuridine monomers in place of the bicyclic nucleoside **4**, were determined. As can be seen when comparing the natural DNA 9-mer in entry 1 with entries 2 and 4, small decreases in the thermal stability result from substituting the natural thymine bases with uracil, most pronouncedly for the 9-mer with one uracil nucleobase when hybridized to complementary DNA. Incorporation of **4** once into the center of the 9-mer (entry 3) effected a decrease in the duplex stability of −2.1 °C toward complementary DNA and −0.8 °C toward complementary RNA. When **4** was incorporated three times in the sequence (entry 5), even larger decreases of −2.9 and −1.5 °C per modification toward complementary DNA and RNA, respectively, were observed. This moderate decrease in duplex stabilities can probably be ascribed to unfavorable steric interactions of the additional six-membered ring combined with the O4′-endo furanose conformation adopted by **4**, which is not quite as favored for duplex formation as the C3′-endo conformation of the structurally closely related bicycle **2** (Figure 1).

Conclusion

In conclusion, the nucleoside **15** of a novel C2′−C5′-fused bicyclic structure was synthesized in several steps

(25) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.

(26) Goodman, J. M.; Still, W. C. *J. Comput. Chem.* **1991**, *12*, 1110−1117.

(27) Halgren, T. A. *J. Comput. Chem.* **1996**, *17*, 490−519. Planar sp^2-hybridized nitrogens were chosen.

(28) Haasnoot, C. A. G.; de Leeuw, A. A.; de Leeuw, H. P. M.; Altona, C. *Org. Magn. Reson.* **1981**, *15*, 43−51.

from 1-(3′-deoxy-β-D-psicofuranosyl)uracil (**5**). Molecular modeling supported the assumption of a fixed O4′-endo furanose conformation. The phosphoramidite derivative **17** was incorporated successfully one and three times into mixed 9-mer oligodeoxynucleotides by standard phosphoramidite oligonucleotide synthesis. Thermal denaturation studies revealed moderate decreases in duplex stabilities of −2.1 and −1.5 °C per modification toward complementary DNA and RNA, respectively. These results underline the fact that a locked furanose conformation is not per se leading to increased duplex stabilities.

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 the 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 (unless otherwise stated), 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 the internal standard for ^{1}H and ^{13}C and relative to 85% H_3PO_4 as the 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. For compound **12**, a long-range HMBC spectrum gave a complete assignment of all isolated methylene groups, while the assignments of atoms 6′ and 6″ may be interchanged for compounds **9**−**11** and **13**−**16**. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode.

1-[1′,4′-Di-*O*-benzyl-3′-deoxy-6′-*O*-(4,4′-dimethoxytrityl)-β-D-psicofuranosyl]uracil (7). Nucleoside **6**[23] (945 mg, 1.69 mmol) was dissolved in anhydrous THF (20 mL) at 0 °C, NaH (60% w/w, 356 mg, 8.9 mmol) was added, and the mixture was stirred at 0 °C for 5 min. BnBr (0.44 mL, 3.70 mmol) was added dropwise, and the mixture was stirred at room temperature for 8 h. H_2O (50 mL) and saturated aq $NaHCO_3$ (50 mL) were added, and the mixture was extracted with EtOAc (4 × 60 mL). The combined organic phase was washed with H_2O (2 × 50 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (3 × 50 mL). The crude residue was dissolved in anhydrous THF (25 mL), NaH (60% w/w, 495 mg, 12.4 mmol) was added, and the mixture was stirred for 5 min. BnBr (0.12 mL, 1.00 mmol) was added dropwise, and the mixture was stirred at room temperature for 15 h. H_2O (50 mL) and saturated aq $NaHCO_3$ (50 mL) were added, and the mixture was extracted with EtOAc (4 × 60 mL). The combined organic phase was washed with H_2O (2 × 50 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (3 × 50 mL). The residue was purified by silica gel column chromatography (16 cm × 2.8 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 **7** (1.055 g, 85%) as a white foam after coevaporation with acetonitrile (3 × 30 mL): R_f (8% MeOH in CH_2Cl_2 (v/v)) 0.49. FAB-MS *m*/*z*: 740.5 $[M]^+$, 741.5 $[M + H]^+$. ^{1}H NMR ($CDCl_3$): δ 8.97 (1H, br s, NH), 7.86 (1H, d, *J* = 8.4 Hz, H6), 7.36−7.19 (19H, m, Bn, DMT), 6.81−6.78 (4H, m, DMT), 5.53 (1H, dd, *J* = 2.3, 8.4 Hz, H5), 4.60 (1H, d, *J* = 12.3 Hz, Bn), 4.50 (1H, d, *J* = 11.9 Hz, Bn), 4.45 (1H, d, *J* = 12.3 Hz, Bn), 4.44 (1H, d, *J* = 11.7 Hz, Bn), 4.44 (1H, m, H5′), 4.07 (1H, dt, *J* = 2.6, 5.9 Hz, H4′), 4.00 (1H, d, *J* = 10.6 Hz, H1′), 3.84 (1H, d, *J* = 10.4 Hz, H1′), 3.78 (6H, s, OMe), 3.20 (2H, d, *J* = 4.6 Hz, H6′), 2.76 (1H, dd, *J* = 6.1, 15.1 Hz, H3′), 2.65 (1H, dd, *J* = 2.6, 15.0 Hz, H3′). ^{13}C NMR ($CDCl_3$): δ 163.66 (C4), 158.46 (DMT), 149.82 (C2), 144.16 (DMT), 142.29 (C6), 135.18, 129.77, 128.35, 128.22, 127.76, 127.74, 127.69, 127.61, 127.47, 127.42, 126.85 (DMT, Bn), 113.03 (DMT), 99.94 (C5), 98.93 (C2′), 86.54 (Ar_2PhC), 85.34 (C5′), 78.84 (C4′), 73.42 (Bn), 71.71 (C1′), 71.03 (Bn), 62.90 (C6′), 55.09 (OMe), 41.11 (C3′).

1-[1′,4′-Di-*O*-benzyl-3′-deoxy-β-D-psicofuranosyl]uracil (8). Compound **7** (2.688 g, 3.68 mmol) was dissolved in CH_2Cl_2 (10 mL), 80% AcOH (50 mL, v/v) was added, and the mixture was stirred for 1 h 30 min and evaporated to an oil under reduced pressure. EtOH (5 mL) and saturated aq $NaHCO_3$ (10 mL) were added, and the suspension was evaporated to dryness under reduced pressure and coevaporated with abs EtOH (2 × 50 mL). The residue was suspended in MeOH (50 mL), evaporated on silica gel, and purified by silica gel column chromatography (7.5 cm × 5.5 cm), eluting with a gradient of 2−5% MeOH in CH_2Cl_2 (v/v) to give nucleoside **8** (1.387 g, 94%) as a white foam after coevaporation with acetonitrile (50 mL): R_f (8% MeOH in CH_2Cl_2 (v/v)) 0.36. ^{1}H NMR ($CDCl_3$): δ 9.05 (1H, br s, NH), 7.95 (1H, d, *J* = 8.2 Hz, H6), 7.36−7.19 (10H, m, Bn), 5.62 (1H, dd, *J* = 1.8, 8.2 Hz, H5), 4.59 (1H, d, *J* = 12.3 Hz, Bn), 4.53 (1H, d, *J* = 11.7 Hz, Bn), 4.46 (1H, d, *J* = 11.7 Hz, Bn), 4.45 (1H, d, *J* = 12.3 Hz, Bn), 4.37 (1H, m, H5′), 4.12 (1H, dt, *J* = 3.1, 6.2 Hz, H4′), 3.99 (1H, d, *J* = 10.4 Hz, H1′), 3.83 (1H, d, *J* = 10.6 Hz, H1′), 3.76 (1H, dd, *J* = 3.8, 11.5 Hz, H6′), 3.65 (1H, dd, *J* = 3.9, 11.4 Hz, H6′), 2.78 (1H, dd, *J* = 6.4, 15.0 Hz, H3′), 2.62 (1H, dd, *J* = 2.9, 15.0 Hz, H3′), 2.42 (1H, br s, OH). ^{13}C NMR ($CDCl_3$): δ 164.09 (C4), 149.76 (C2), 142.44 (C6), 137.43, 137.37, 128.37, 128.25, 127.74, 127.64, 127.49, 127.43 (Bn), 99.77 (C5), 98.93 (C2′), 86.44 (C5′), 78.44 (C4′), 73.45 (Bn), 71.56 (C1′), 71.23 (Bn), 62.30 (C6′), 41.06 (C3′).

1-[1′,4′-Di-*O*-benzyl-3′-deoxy-5′-*C*-hydroxymethyl-β-D-psicofuranosyl]uracil (9). A solution of **8** (1.520 g, 3.47 mmol) in anhydrous CH_2Cl_2 (20 mL) was added to a suspension of Dess−Martin periodinane (1.824 g, 4.30 mmol) in anhydrous CH_2Cl_2 (20 mL), and the suspension was stirred for 40 min. CH_2Cl_2 (200 mL) was added, and the mixture was poured into a solution of $Na_2S_2O_3$·5H_2O (4061 mg, 16.36 mmol) in H_2O (100 mL) and swirled until the solid had dissolved. The layers were separated, the organic phase was filtered and evaporated to dryness under reduced pressure, and the residue was dissolved in 1,4-dioxane (100 mL). 37% aq formaldehyde (4.0 mL) and 1 M aq NaOH (20 mL) were added, and the mixture was stirred for 22 h. Saturated aq $NaHCO_3$ (20 mL) was added, and the suspension was evaporated to dryness under reduced pressure, coevaporated with acetonitrile (50 mL), suspended in MeOH (50 mL), and evaporated on silica gel. The residue was purified by silica gel column chromatography (6.5 cm × 5.5 cm), eluting with a gradient of 1−5% MeOH in CH_2Cl_2 (v/v) to give compound **9** (1.274 g, 78%) as a white foam: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.33. FAB-MS *m*/*z*: 469.1 $[M + H]^+$. ^{1}H NMR ($CDCl_3$): δ 9.12 (1H, br s, NH), 7.98 (1H, d, *J* = 8.2 Hz, H6), 7.36−7.20 (10H, m, Bn), 5.63 (1H, d, *J* = 8.2 Hz, H5), 4.59 (1H, d, *J* = 11.5 Hz, Bn), 4.57 (1H, d, *J* = 12.1 Hz, Bn), 4.47 (1H, d, *J* = 12.1 Hz, Bn), 4.39 (1H, d, *J* = 11.7 Hz, Bn), 4.17 (1H, dd, *J* = 5.1, 6.4 Hz, H4′), 3.87 (1H, d, *J* = 11.5 Hz, H1′), 3.86 (2H, s, H6″), 3.80 (1H, d, *J* = 10.3 Hz, H1′), 3.68 (1H, d, *J* = 11.7 Hz, H6′), 3.64 (1H, d, *J* = 11.7 Hz, H6′), 3.05 (1H, dd, *J* = 6.5, 14.9 Hz, H3′), 2.68 (1H, dd, *J* = 5.0, 14.9 Hz, H3′). ^{13}C NMR ($CDCl_3$): δ 164.08 (C4), 149.95 (C2), 142.16 (C6), 137.17, 137.04, 128.50, 128.33, 127.98, 127.81, 127.61, 127.39 (Bn), 100.06 (C5), 98.17 (C2′), 89.80 (C5′), 79.05 (C4′), 73.52 (Bn), 71.96, 71.90 (Bn, C1′), 63.89 (C6′), 63.06 (C6″), 39.36 (C3′).

1-[1′,4′-Di-*O*-benzyl-3′-deoxy-5′-*C*-methanesulfonyloxymethyl-6′-*O*-methanesulfonyl-β-D-psicofuranosyl]uracil (10). Nucleoside **9** (1.000 g, 2.13 mmol) was dissolved in anhydrous pyridine (80 mL), evaporated to approximately half the volume, and cooled to 0 °C. MsCl (0.70 mL, 9.0 mmol) was added, and the mixture was stirred for 1 h 20 min at 0 °C. Saturated aq $NaHCO_3$ (50 mL) and H_2O (50 mL) were added, and the suspension was extracted with EtOAc (4 × 50 mL). The combined organic phase was washed with H_2O (50 mL),

evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (2 × 50 mL). The residue was purified by silica gel column chromatography (5.7 cm × 5.5 cm), eluting with a gradient of 40−80% EtOAc in petroleum ether (v/v) to give compound **10** (1.146 g containing *n*-hexane as an impurity) as a white foam: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.57. FAB-MS *m*/*z*: 625.6 $[M + H]^+$. ^{1}H NMR ($CDCl_3$): δ 9.2 (1H, br s, NH), 7.77 (1H, d, J = 8.2 Hz, H6), 7.37−7.20 (10H, m, Bn), 5.68 (1H, d, J = 8.2 Hz, H5), 4.60 (1H, d, J = 11.5 Hz, Bn), 4.55 (1H, d, J = 12.1 Hz, Bn), 4.52 (1H, d, J = 12.6 Hz, H6′), 4.47 (1H, d, J = 12.1 Hz, Bn), 4.40 (2H, d, J = 11.2 Hz, Bn, H6′), 4.25−4.18 (3H, m, H4′, H6″), 3.90 (1H, d, J = 10.6 Hz, H1′), 3.79 (1H, d, J = 10.4 Hz, H1′), 3.02 (1H, m, H3′), 2.99 (3H, s, Ms), 2.93 (3H, s, Ms), 2.79 (1H, dd, J = 3.6, 15.4 Hz, H3′). ^{13}C NMR ($CDCl_3$): δ 163.59 (C4), 149.83 (C2), 141.52 (C6), 137.09, 136.34, 128.55, 128.36, 128.19, 127.85, 127.71, 127.54 (Bn), 100.67 (C5), 98.79 (C2′), 86.52 (C5′), 78.57 (C4′), 73.46 (Bn), 72.10 (Bn), 71.73 (C1′), 67.91 (C6′), 66.58 (C6″), 39.63 (C3′), 37.51, 37.23 (Ms). *n*-Hexane was assigned as an impurity.

1-[4′-*O*-Benzyl-3′-deoxy-5′-*C*-methanesulfonyloxymethyl-6′-*O*-methanesulfonyl-*β*-D-psicofuranosyl]uracil (11) and 1-[3′-Deoxy-5′-*C*-methanesulfonyloxymethyl-6′-*O*-methanesulfonyl-*β*-D-psicofuranosyl]uracil (11B). Compound **10** (463 mg) was dissolved in anhydrous CH_2Cl_2 (2.5 mL); anhydrous EtOH (25 mL) and $Pd(OH)_2$/C (233 mg, 20% w/w) were added, and the mixture was evacuated with H_2 several times. The suspension was stirred under an atmosphere of hydrogen for 7 h, evaporated to dryness under pressure, resuspended in CH_2Cl_2 (50 mL), and evaporated on silica gel. The residue was purified by silica gel column chromatography (10.3 cm × 2.8 cm), eluting with a gradient of 1−4% MeOH in CH_2Cl_2 (v/v) to give mono- and didebenzylated nucleosides **11** (252 mg, 55% from **9**) and **11B** (43 mg, 11% from **9**), respectively, as white foams. Physical data for **11**: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.46. FAB-MS *m*/*z*: 535.4 $[M + H]^+$. ^{1}H NMR ($CDCl_3$): δ 9.9 (1H, br s, NH), 7.77 (1H, d, J = 8.2 Hz, H6), 7.36−7.26 (5H, m, Bn), 5.61 (1H, d, J = 8.3 Hz, H5), 4.63 (1H, d, J = 11.8 Hz, H6′), 4.60 (1H, d, J = 12.0 Hz, Bn), 4.38 (1H, d, J = 11.5 Hz, H6′), 4.37 (1H, d, J = 11.8 Hz, Bn), 4.28 (1H, d, J = 10.6 Hz, H6″), 4.26 (1H, br s, H4′), 4.20 (1H, d, J = 10.4 Hz, H6″), 4.01 (1H, d, J = 12.3 Hz, H1′), 3.85 (1H, d, J = 12.2 Hz, H1′), 3.25 (1H, br s, OH), 3.04 (3H, s, Ms), 3.02 (1H, m, H3′), 3.00 (3H, s, Ms), 2.86 (1H, d, J = 14.1 Hz, H3′). ^{13}C NMR ($CDCl_3$): δ 164.42 (C4), 150.08 (C2), 142.08 (C6), 136.43, 128.54, 128.19, 127.83 (Bn), 100.63 (C5), 99.84 (C2′), 86.34 (C5′), 79.03 (C4′), 71.89 (Bn), 68.27 (C6′), 66.44 (C6″), 65.42 (C1′), 39.05 (C3′), 37.63, 37.37 (Ms). Physical data for **11B**: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.26. FAB-MS *m*/*z*: 445.2 $[M + H]^+$. ^{1}H NMR (CD_3OD): δ 7.97 (1H, d, J = 8.2 Hz, H6), 5.69 (1H, d, J = 8.2 Hz, H5), 4.56 (2H, s, H6′), 4.43 (1H, dd, J = 2.6, 6.1 Hz, H4′), 4.36 (1H, d, J = 10.6 Hz, H6″), 4.32 (1H, d, J = 10.6 Hz, H6″), 3.92 (2H, s, H1″), 3.23 (3H, s, Ms), 3.17 (3H, s, Ms), 3.08 (1H, dd, J = 6.1, 15.3 Hz, H3′), 2.64 (1H, dd, J = 2.1, 15.3 Hz, H3′). ^{13}C NMR (CD_3OD): δ 166.94 (C4), 151.88 (C2), 143.74 (C6), 101.42, 101.13 (C5, C2′), 88.92 (C5′), 73.18 (C4′), 69.52, 69.17 (C6′, C6″), 66.14 (C1′), 44.73 (C3′), 37.43 (Ms).

(1*S*,5*R*,6*S*)-6-Benzyloxy-5-methanesulfonyloxymethyl-1-(uracil-1-yl)-3,8-dioxabicyclo[3.2.1]octane (12). Nucleoside **11** (378 mg, 0.707 mmol) was dissolved in 1,4-dioxane (10 mL) and 1 M aq NaOH (5 mL), and the mixture was stirred at 90 °C for 22 h. After cooling, saturated aq $NaHCO_3$ (20 mL) and H_2O (20 mL) were added, and the mixture was extracted with EtOAc (4 × 25 mL) and CH_2Cl_2 (2 × 15 mL). The combined organic phase was washed with H_2O (2 × 20 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (25 mL). The residue was purified by silica gel column chromatography (10 cm × 2.8 cm), eluting with a gradient of 1−3% MeOH in CH_2Cl_2 (v/v) to give bicycle **12** (197 mg, 64%) as a white solid: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.51. FAB-MS *m*/*z*: 439.1 $[M + H]^+$. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.35 (1H, br s, NH), 7.63 (1H, d, J = 7.8 Hz, H6), 7.39−7.29 (5H, m, Bn), 5.63 (1H, d, J = 8.3 Hz, H5), 4.60 (1H, d, J = 12.2 Hz, Bn), 4.55 (1H, d, J = 11.7 Hz, Bn), 4.37 (1H, d, J = 11.7 Hz, H6′), 4.32 (1H, d, J = 11.7 Hz, H6′), 4.10 (1H, dd, J = 4.2, 10.5 Hz, H4′), 4.05 (1H, d, J = 10.7 Hz, H1′), 3.94 (1H, d, J = 11.2 Hz, H6″), 3.58 (1H, d, J = 11.2 Hz, H6″), 3.36 (1H, d, J = 10.3 Hz, H1′), 3.23 (3H, s, Ms), 2.66 (1H, dd, J = 4.4, 13.0 Hz, H3′), 2.48 (1H, dd, J = 10.3, 13.4 Hz, H3′). ^{13}C NMR (126 MHz, DMSO-d_6): δ 163.24 (C4), 149.57 (C2), 139.33 (C6), 137.99, 128.29, 127.63 (Bn), 101.27 (C5), 91.87 (C2′), 82.89 (C5′), 76.97 (C4′), 71.95 (Bn), 70.30 (C1′), 68.43 (C6′), 64.99 (C6″), 40.98 (C3′), 36.82 (Ms).

(1*S*,5*S*,6*S*)-6-Benzyloxy-5-hydroxymethyl-1-(uracil-1-yl)-3,8-dioxabicyclo[3.2.1]octane (14). Bicycle **12** (197 mg, 0.45 mmol) was dissolved in anhydrous DMF (15 mL), NaOBz (364 mg, 2.53 mmol) was added, and the suspension was stirred at 120 °C for 36 h. Saturated aq $NaHCO_3$ (15 mL) and H_2O (15 mL) were added, and the mixture was extracted with EtOAc (4 × 25 mL). The combined organic phase was washed with H_2O (15 mL), evaporated to dryness under reduced pressure, and coevaporated with acetonitrile (2 × 25 mL) and *n*-hexane/CH_2Cl_2 (5 × 50 mL, 4:1 (v/v)). Crude **13** (see below) was dissolved in MeOH (25 mL), NaOMe (230 mg, 4.3 mmol) was added, and the mixture was stirred for 1 h 30 min. Saturated aq $NaHCO_3$ (1 mL) was added; the suspension was evaporated to dryness under reduced pressure, suspended in CH_2Cl_2 (50 mL), and evaporated on silica gel. The residue was purified by silica gel column chromatography (10 cm × 2.8 cm), eluting with a gradient of 1−3% MeOH in CH_2Cl_2 (v/v) to give nucleoside **14** (145 mg, 90%) as a white foam: R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.33. FAB-MS *m*/*z*: 361.1 $[M + H]^+$. ^{1}H NMR (1:1 $CDCl_3$/CD_3OD (v/v)): δ 7.82 (1H, d, J = 8.2 Hz, H6), 7.29−7.22 (5H, m, Bn), 5.61 (1H, d, J = 8.2 Hz, H5), 4.62 (1H, d, J = 11.9 Hz, Bn), 4.47 (1H, d, J = 11.9 Hz, Bn), 4.20 (1H, d, J = 10.2 Hz, H1′), 3.99 (1H, d, J = 11.5 Hz, H6″), 3.98 (1H, m, H4′), 3.63 (1H, d, J = 12.6 Hz, H6′), 3.61 (1H, d, J = 11.5 Hz, H6″), 3.49 (1H, d, J = 12.4 Hz, H6′), 3.30 (1H, d, J = 10.4 Hz, H1′), 2.88 (1H, dd, J = 4.1, 13.7 Hz, H3′), 2.35 (1H, dd, J = 10.4, 13.7 Hz, H3′). ^{13}C NMR (1:1 $CDCl_3$/CD_3OD (v/v)): δ 165.40 (C4), 150.47 (C2), 141.03 (C6), 138.28, 128.90, 128.33, 128.22 (Bn), 101.66 (C5), 92.81 (C2′), 86.33 (C5′), 77.65 (C4′), 73.21 (Bn), 71.53 (C1′), 66.90 (C6″), 62.49 (C6″), 42.11 (C3′).

(1*S*,5*R*,6*S*)-6-Benzyloxy-5-benzoyloxymethyl-1-(uracil-1-yl)-3,8-dioxabicyclo[3.2.1]octane (13). Physical data of the intermediary benzoate **13** purified in analytical scale by silica gel column chromatography, eluting with a gradient of 0−10:100−90 acetone in CH_2Cl_2 (v/v): R_f (20% acetone in CH_2Cl_2 (v/v)) 0.55. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.74 (1H, br s, NH), 7.95 (2H, dd, J = 1.4, 8.1 Hz, Bz), 7.66 (1H, dd, J = 1.2, 8.1 Hz, H6), 7.61−7.24 (8H, m, Bn, Bz), 5.69 (1H, d, J = 8.1 Hz, H5), 4.75 (1H, d, J = 12.0 Hz, Bn), 4.48 (2H, d, J = 12.3 Hz, Bn, H6′), 4.32 (1H, d, J = 10.8 Hz, H1′), 4.28 (1H, d, J = 12.3 Hz, H6′), 4.20 (1H, d, J = 11.1 Hz, H6″), 4.09 (1H, dd, J = 4.2, 10.2 Hz, H4′), 3.72 (1H, d, J = 11.4 Hz, H6″), 3.37 (1H, d, J = 10.5 H1′), 3.05 (1H, dd, J = 4.2, 13.8 Hz, H3′), 2.44 (1H, dd, J = 10.5, 13.5 Hz, H3′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.66 (O*C*OPh), 163.61 (C4), 149.38 (C2), 139.45 (C6), 137.03 (Bn), 133.26 (Bz), 129.49, 129.09, 128.33, 128.31, 127.81, 127.65 (Bn, Bz), 101.55 (C5), 92.33 (C2′), 83.76 (C5′), 76.77 (C4′), 72.42 (Bn), 70.84 (C1′), 66.15 (C6′), 63.43 (C6″), 40.98 (C3′).

(1*S*,5*S*,6*S*)-6-Hydroxy-5-hydroxymethyl-1-(uracil-1-yl)-3,8-dioxabicyclo[3.2.1]octane (15). Compound **14** (116 mg, 0.322 mmol) was dissolved in anhydrous CH_2Cl_2 (25 mL) at −78 °C, BCl_3 (3.0 mL, 1 M solution in hexanes, 3.0 mmol) was added dropwise over the course of 15 min, and the mixture was stirred at −78 °C for 7 h and at room temperature for 30 min. After cooling to −78 °C, MeOH (10 mL) was added, and the mixture was stirred at room temperature overnight. The mixture was evaporated to dryness under reduced pressure and coevaporated with MeOH (3 × 10 mL). The residue was purified by silica gel column chromatography (8.5 cm × 2.0 cm), eluting with a gradient of 5−10% MeOH in CH_2Cl_2 (v/v) to give nucleoside **15** (73 mg, 84%) as a white solid: R_f (20% MeOH in CH_2Cl_2 (v/v)) 0.51. FAB-MS *m*/*z*: 271.0 $[M + H]^+$. Found: *m*/*z* (FAB, NBA + PEG300 matrix) 271.0930. Calcd for $C_{11}H_{15}O_6N_2$: 271.0930. ^{1}H NMR (DMSO-d_6): δ 11.29 (1H, s, NH), 7.76 (1H, d, J = 8.2 Hz, H6), 5.60 (1H, d, J = 8.1 Hz,

H5), 5.23 (1H, d, J = 5.3 Hz, OH-4′), 4.87 (1H, t, J = 6.0 Hz, OH-6′), 4.02 (1H, d, J = 10.1 Hz, H1′), 4.01 (1H, m, H4′), 3.84 (1H, d, J = 11.2 Hz, H6″), 3.53 (1H, dd, J = 5.9, 12.3 Hz, H6′), 3.50 (1H, d, J = 11.2 Hz, H6″), 3.39 (1H, dd, J = 6.0, 12.5 Hz, H6′), 3.23 (1H, d, J = 10.3 Hz, H1′), 2.50 (1H, m, H3′), 2.37 (1H, dd, J = 10.9, 13.5 Hz, H3′). ^{13}C NMR (DMSO-d_6): δ 163.43 (C4), 149.64 (C2), 139.93 (C6), 100.78 (C5), 91.28 (C2′), 85.85 (C5′), 70.50 (C1′), 69.54 (C4′), 65.54 (C6″), 61.39 (C6′), 43.56 (C3′).

(1*S*,5*R*,6*S*)-5-(4,4′-Dimethoxytrityloxymethyl)-6-hydroxy-1-(uracil-1-yl)-3,8-dioxabicyclo[3.2.1]octane (16). Nucleoside **15** (73 mg, 0.269 mmol) was dissolved in anhydrous pyridine (10 mL), evaporated to about half the volume under reduced pressure, and cooled to 0 °C, whereupon DMTCl (184 mg, 0.541 mmol) was added. The mixture was stirred at room temperature for 2 h 20 min, additional DMTCl (93 mg, 0.27 mmol) was added, and the mixture was stirred for 30 min. MeOH (1 mL) and saturated aq $NaHCO_3$ (1 mL) were added, and the mixture was evaporated to dryness under reduced pressure, coevaporated with acetonitrile (2 × 30 mL), suspended in CH_2Cl_2 (20 mL), and evaporated on silica gel. The residue was purified by silica gel column chromatography (9.8 cm × 2.0 cm), eluting with a gradient of 0.5:0−3:99.5−96.5 pyridine/MeOH/CH_2Cl_2 (v/v/v) to give nucleoside **16** (103 mg, 67%) as a white foam after coevaporation with acetonitrile (5 × 10 mL) and CH_2Cl_2 (10 mL): R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.43. FAB-MS m/z: 573.1 $[M + H]^+$. ^{1}H NMR (99:1 $CDCl_3$/CD_3OD (v/v)): δ 10.05 (1H, br s, NH), 7.63 (1H, d, J = 8.2 Hz, H6), 7.36−7.14 (9H, m, DMT), 6.77−6.74 (4H, m, DMT), 5.64 (1H, d, J = 8.2 Hz, H5), 4.23 (1H, d, J = 10.3 Hz, H1′), 4.04 (1H, m, H4′), 4.03 (1H, d, J = 11.7 Hz, H6″), 3.79 (1H, d, J = 11.7 Hz, H6″), 3.71 (6H, s, OMe), 3.32 (1H, d, J = 10.3 Hz, H1′), 3.18 (2H, s, H6′), 2.71 (1H, dd, J = 3.8, 14.1 Hz, H3′), 2.56 (1H, br s, OH), 2.44 (1H, dd, J = 11.2, 14.1 Hz, H3′). ^{13}C NMR (99:1 $CDCl_3$/CD_3OD (v/v)): δ 163.75 (C4), 158.35 (DMT), 149.45 (C2), 144.15 (DMT), 139.53 (C6), 135.31, 135.16, 129.85, 129.79, 127.96, 127.83, 127.64, 127.48, 126.73, 112.96, 112.80 (DMT), 101.38 (C5), 91.77 (C2′), 86.09 (*C*Ar$_2$Ph), 85.56 (C5′), 72.33 (C4′), 71.19 (C1′), 67.30 (C6″), 64.25 (C6′), 55.00 (OMe), 44.27 (C3′).

(1*S*,5*R*,6*S*)-6-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-5-(4,4′-dimethoxytrityloxymethyl)-1-(uracil-1-yl)-3,8-dioxabicyclo[3.2.1]octane (17). Nucleoside **16** (103 mg, 0.179 mmol) was coevaporated with anhydrous acetonitrile (2 × 10 mL), dissolved in anhydrous CH_2Cl_2 (5 mL), and cooled to 0 °C. Diisopropylethylamine (0.5 mL) was added, followed by 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.25 mL, 1.1 mmol), and the mixture was stirred at room temperature for 3 h. H_2O (2 mL) and EtOAc (100 mL) were added, and the mixture was washed with saturated aq $NaHCO_3$ (20 mL) and H_2O (20 mL). The organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (2 × 20 mL). The residue was purified twice by silica gel column chromatography, eluting first with 0.5:50:49.5 pyridine/EtOAc/*n*-hexane (v/v/v) and then with a gradient of 0.5:25−60:74.5−39.5 pyridine/EtOAc/*n*-hexane (v/v/v) to give phosphoramidite **17** (36 mg, 26%) as a white foam after coevaporation with acetonitrile (4 × 5 mL) and CH_2Cl_2 (5 mL): R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.50. FAB-MS m/z: 773.2 $[M + H]^+$. ^{31}P NMR ($CDCl_3$): δ 150.59.

Oligonucleotide Synthesis. All oligomers were synthesized on a DNA synthesizer using the phosphoramidite approach.[29] The stepwise coupling yields were determined spectrophotometrically at 498 nm (quantifying the released 4,4′-dimethoxytrityl group). Standard conditions were used, except for the phosphoramidite **17** which 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 10 min]. Coupling yields for the amidite **17** were ∼98%, while coupling yields for the unmodified amidites were >99%. Cleavage from the solid support and removal of the protecting groups was accomplished using conc aq ammonia (55 °C for 16 h), and purification, by ethanol precipitation of the oligonucleotides. Capillary gel electrophoresis of all synthesized sequences showed >90% purity. The composition of the modified 9-mers was verified by MALDI-MS analysis. Found (entry 3, Table 1): m/z 2778.6 $[M - H]^-$. Calcd: 2780.7. Found (entry 5, Table 5): m/z 2833.6 $[M - H]^-$. Calcd: 2836.4.

Acknowledgment. Ms. Britta Dahl, Department of Chemistry, University of Copenhagen, is thanked for oligonucleotide synthesis. Dr. Michael Meldgaard, Exiqon A/S, is thanked for recording the MALDI-MS spectra. Dr. Per-Ola Norrby, Department of Chemistry, Technical University of Denmark, is thanked for the molecular modeling. The Danish Natural Science Research Council, The Danish Technical Research Council, and Exiqon A/S are thanked for financial support.

Supporting Information Available: Copies of ^{13}C NMR spectra for **7**−**11**, **11B**, and **12**−**16**, a copy of the ^{31}P NMR spectrum for **17**, copies from the transcript from capillary gel electrophoretic analysis of the oligonucleotides containing **4** (entries 3 and 5, Table 1), and Cartesian coordinates of the lowest-energy conformer of **15** obtained by the energy-minimization (15.cc1). This material is available free of charge via the Internet at http://pubs.acs.org.

JO015664L

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