Convenient Syntheses of 7-Hydroxy-1-(hydroxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptanes: α-L-Ribo- and α-L-Xylo-Configured LNA Nucleosides

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Synthesis of the diastereoisomeric LNA (locked nucleic acid) nucleosides 1-(2-*O*,4-*C*-methylene-α-L-ribofuranosyl)thymine (**6**) and 1-(2-*O*,4-*C*-methylene-α-L-xylofuranosyl)thymine (**13**) are reported via convenient reaction cascades from di-*O*-*p*-toluenesulfonyl and tri-*O*-methanesulfonyl nucleoside derivatives **3**, **7**, and **10**.

"LNA" (locked nucleic acid, β-D-ribo isomer, Figure 1) is a novel nucleic acid analogue[1−3] displaying unprecedented binding affinity toward complementary DNA and RNA. The furanose ring of an LNA nucleoside monomer, being part of a dioxabicyclo[2.2.1]heptane skeleton, is efficiently locked in an N-type conformation (C3′-*endo*/^3E conformation). We have very recently studied the properties of diastereoisomeric forms of LNA (Figure 1), i.e. "α-L-LNA" (α-L-ribo isomer),[4,5] "xylo-LNA" (β-D-xylo isomer),[4] and "α-L-xylo-LNA" (α-L-xylo isomer).[5] Especially the fact[4,5] that α-L-LNA displays nucleic acid binding properties closely approaching those of parent LNA highlights the importance of developing efficient synthetic procedures for the diastereoisomeric LNA nucleosides. Whereas synthesis of the monomeric thymine LNA and xylo-LNA nucleosides has been reported earlier,[1,6] we herein describe the first syntheses of the thymine α-L-LNA and α-L-xylo-LNA nucleosides **6** and **13**, respectively.

The starting material for synthesis of the α-L-LNA thymine nucleoside **6** (Scheme 1) was the 4′-*C*-hydroxymethyl nucleoside **1** which was obtained from 1,2:5,6-di-*O*-isopropylidine-α-D-glucofuranose as described earlier.[6] Selective monoprotection of **1** by reaction with 1.1 equiv of 4,4′-dimethoxytrityl chloride ((DMT)Cl) afforded nucleoside diol **2** in limited 31% yield as the major product. According to analytical TLC, a slightly more polar DMT-containing byproduct as well as a less polar DMT-containing byproduct were formed in minor amounts. The preferential formation of the desired mono DMT-protected product **2** can be explained by sterical hindrance, i.e. the positioning of both a benzyloxy and a thymine moiety at the same face of the furanose ring in starting material **1**. The structure of **2** was conclusively assigned on the basis of the unambiguous structure of the cyclized nucleosides, e.g. **4** and **6**. To prepare for ring closure, the di-*O*-tosylated derivative **3** was prepared in 63% yield by treatment with a 10-fold excess of *p*-toluenesulfonyl chloride (TsCl) and a catalytic amount of 4-(*N*,*N*-dimethylamino)pyridine (DMAP) in pyridine. Conversion of nucleoside **3** directly to the desired 2,5-dioxabicyclo-[2.2.1]heptane derivative **4** proceeded very satisfactory in 81% yield in a mixture of 2 M aqueous NaOH and H_2O:EtOH (1:1; heating under reflux for 24 h). This conversion involves epimerization at the 2′-carbon atom and subsequent cyclization and is anticipated to proceed via the three integrated reaction steps shown in Figure 2. Thus,

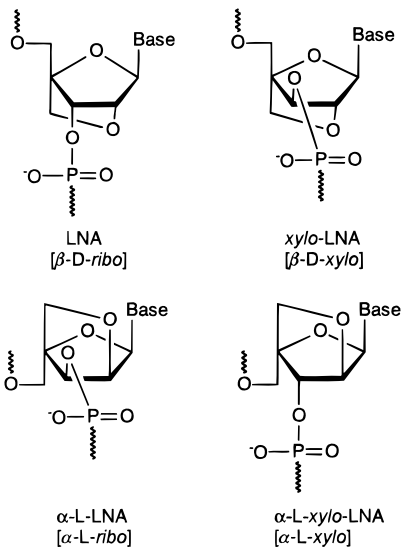

Figure 1. Four synthesized diastereoisomeric LNAs.[1−5]

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(2) We have defined LNA as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-β-D-ribofuranosyl nucleotide monomer(s). The natural β-D-ribo configuration is assigned to LNA (and LNA nucleosides) as the positioning of the 1-nitrogen and 2′-, 3′- and 5′-oxygen atoms follows that of RNA.

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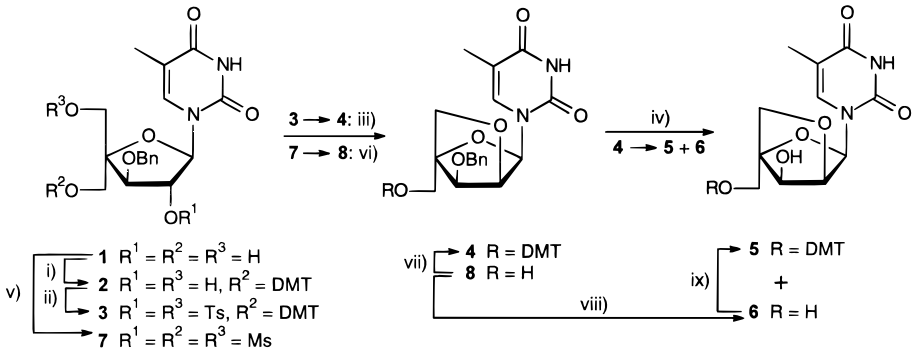
Scheme 1[a]



[a] *Reagents:* (i) DMTCl, $AgNO_3$, THF, pyridine (31%); (ii) TsCl, DMAP, pyridine (63%); (iii) 2 M NaOH, EtOH, H_2O (81%); (iv) $HCOONH_4$, 10% Pd/C, MeOH (**5**: 80%; **6**: 17%); (v) MsCl, pyridine (92%); (vi) 6 M NaOH, EtOH, H_2O (58%); (vii) DMTCl, pyridine (93%); (viii) H_2/($Pd(OH)_2$/C)/EtOH (98%); (ix) DMTCl, pyridine (98%).

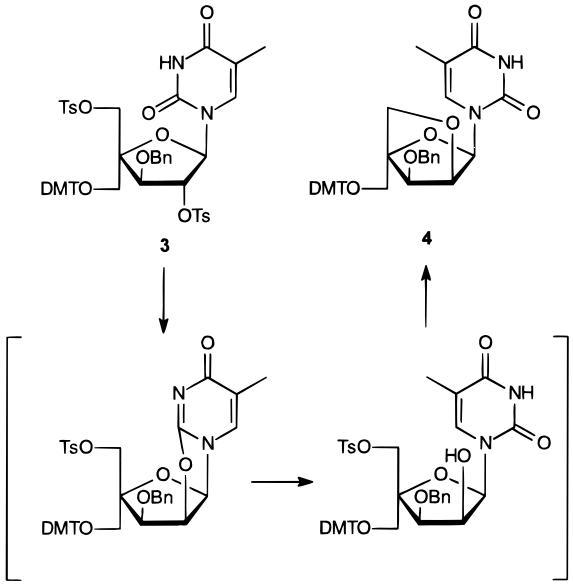


Figure 2. Suggested mechanism for the conversion of nucleoside **3** into the bicyclic derivative **4**.

a reaction cascade involving 2-2′-anhydro intermediate formation by intramolecular nucleophilic attack, hydrolysis of this intermediate to give a putative 2′-OH intermediate with inverted configuration at the 2′-carbon atom, and a second intramolecular nucleophilic attack on the 5′-tosyloxy group from the inverted 2′-OH group offers a probable mechanism for the successful stereoselective conversion of compound **3** to **4**. This convenient synthesis of nucleoside **4** stresses the usefulness of anhydro-intermediate formation[7] in pyrimidine nucleoside chemistry as also illustrated by recent syntheses of nucleosides containing other bicyclic furanose moieties.[8,9]

For automated synthesis of α-L-LNA the phosphoramidite approach[10] with 5′-*O*-DMT-protected 3′-*O*-phosphitylated building blocks was used.[4] It was therefore very convenient that hydrogenolysis of nucleoside **4** chemoselectively furnished the debenzylated 5′-*O*-DMT-protected derivative **5** in 80% yield together with a small amount (17% yield) of 1-(2-*O*,4-*C*-methylene-α-L-ribofuranosyl)thymine (**6**), the parent α-L-LNA thymine nucleoside (Scheme 1). Conversion of DMT-derivative **5** to the desired 3′-*O*-phosphitylated building block has been described elsewhere.[4a]

Alternatively, the tri-*O*-mesyl derivative **7** was obtained in 92% yield from nucleoside triol **1** by reaction with excess methanesulfonyl chloride (MsCl) in anhydrous pyridine. Treatment of nucleoside **7** with a mixture of 6 M NaOH and H_2O:EtOH (1:1; heating under reflux for 43 h) afforded the desired bicyclic 3′-*O*-benzylated derivative **8** in 58% yield (Scheme 1). An analogous reaction cascade such as the one described above is anticipated to be involved, in this case supplemented by the conversion of the 5′-mesyloxy group into a hydroxy group.[11] DMT protection of nucleoside **8** afforded in 93% compound **4** which above has been shown to be a convenient intermediate in the synthesis of nucleoside **5**. In another approach toward **5**, debenzylation of nucleoside **8** to give diol **6** was attempted first unsuccessfully using ammonium formate/(10% Pd/C)/MeOH (reflux, 53 h) and then successfully in 98% yield using H_2/($Pd(OH)_2$/C)/EtOH (room temperature, 24 h). Subsequent selective DMT protection of the primary hydroxy group of nucleoside **6** afforded in 98% yield the desired nucleoside intermediate **5** (see above). The two alternative routes from nucleoside **1** to intermediate **5** (via **2**, **3**, and **4** or via **7**, **8**, and **6**) involve the same number of steps but especially the straightforward synthesis of tri-*O*-mesylate **7** from **1** in 92% yield makes this route the preferred one, not only for synthesis of **5** but also for synthesis of the unprotected α-L-LNA nucleoside **6**. It should be mentioned that the cyclization efficiency (see also the subsequent conversion of **10** to **11**) seems comparable for 2′-*O*-tosyl and 2′-O-mesyl derivatives, and, e.g., the tri-*O*-tosyl derivative of **7** would likely afford the 5′-O-tosyl derivative of **8**. However, due to the ease of conversion of a 5′-*O*-mesyl LNA derivative into the desired 5′-OH derivative,[11] we choose to proceed via the tri-*O*-mesyl derivatives **7** and **10**.

In Scheme 2, an analogous approach for synthesis of the α-L-xylo-LNA thymine nucleoside **13** is described starting from 5′-*O*-mesyl-4′-*C*-mesyloxymethyl-D-ribofuranosyl nucleoside **9**.[11] 2′-*O*-Mesylation afforded in 97% yield the tri-*O*-mesyl nucleoside **10**, and concomitant treatment with a mixture of 2 M aqueous NaOH and H_2O:EtOH (1:1; heating under reflux for 40 h) yielded a

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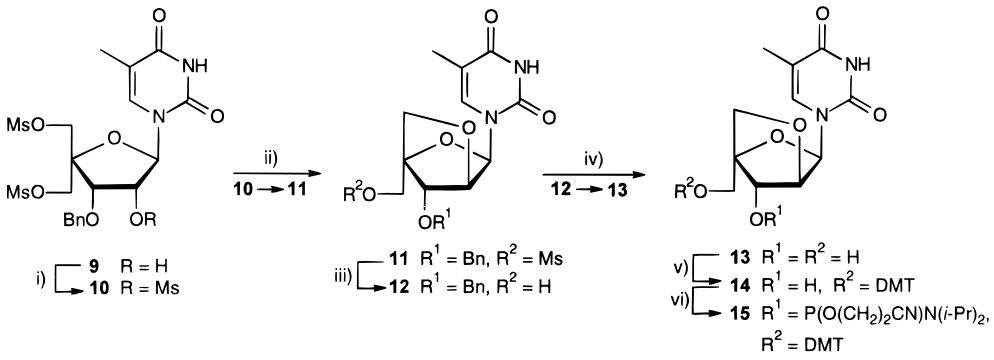
Scheme 2[a]



[a] *Reagents:* (i) MsCl, pyridine (97%); (ii) 2 M NaOH, EtOH, H_2O (**11**: 35%; **12**: 32%); (iii) KOH, EtOH (79%); (iv) H_2, 20% $Pd(OH)_2/C$, EtOH (93%); (v) DMTCl, pyridine (95%); (vi) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2 (60%).

mixture of 5′-*O*-mesyl nucleoside **11** (35% yield) and the desired 5′-hydroxy nucleoside **12** (32% yield). Under more vigorous conditions (KOH in EtOH, 60 °C, 12 h) it was possible to achieve efficient conversion of **11** to **12** (79% yield). Debenzylation of 3′-*O*-benzyl nucleoside **12** to give 1-(2-*O*,4-*C*-methylene-α-L-xylofuranosyl)thymine (**13**), the parent α-L-xylo-LNA thymine nucleoside, proceeded in 93% yield with H_2 and 20% $Pd(OH)_2/C$ in EtOH. To prepare for automated synthesis of α-L-xylo-LNA oligomers, the primary hydroxy group of nucleoside diol **13** was selectively DMT-protected to give nucleoside **14** in 95% yield by reaction with DMTCl in pyridine followed by 3′-*O*-phosphitylation in 60% yield using 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite and *N*,*N*-diisopropylethylamine in CH_2Cl_2, yielding the desired phosphoramidite derivative **15**.[12]

By virtue of their 2,5-dioxabicyclo[2.2.1]heptane skeleton the conformations of the furanose rings of the diastereoisomeric LNA nucleosides **6** and **13** are efficiently locked. Molecular modeling (HyperChem program, Polak-Ribiere algorithm) show that the furanose ring of the α-L-LNA nucleosides **6** and the α-L-xylo-LNA nucleoside **13** adopt an S-type conformation (C3′-*exo*/$_3E$ conformation). For structural verification, NOE experiments were performed on the unprotected nucleosides **6** and **13**. For compound **6**, mutual NOEs between H-6/H-5″ (6%/11%; H-5″ designates the methylene hydrogens in the 4′-*C*-branch), H-1′/H-2′ (9%/8%), and H-1′/H-3′ (17%/11%), as well as NOEs in 3′-OH (2% when irradiating H-5″) and in H-3′ (3% when irradiating H-5′) support the assigned α-L-ribo configuration and C3′-*exo* conformation. For compound **13**, mutual NOEs between H-6/H-5″ (3%/11%) and H-1′/H-2′ (8%/8%), as well as NOEs in 3′-OH (3% when irradiating H-5′) and in H-2′ (3% when irradiating 3′-OH) support the assigned α-L-xylo configuration and C3′-*exo* conformation.

In conclusion, convenient syntheses of the diastereoisomeric LNA nucleosides 1-(2-*O*,4-*C*-methylene-α-L-ribofuranosyl)thymine (**6**) and 1-(2-*O*,4-*C*-methylene-α-L-xylofuranosyl)thymine (**13**), and protected derivatives thereof, have been developed. The syntheses involve efficient reaction cascades from di-*O*-tosyl and tri-*O*-mesyl nucleoside derivatives with stereochemical inversion at the 2′-carbon atoms which limits the synthetic utility to nucleosides of the pyrimidine series. However, the methods devised have allowed discovery of the remarkable hybridization properties of α-L-LNA and should in addition allow synthesis of a variety of novel nucleosides for biological screening.

(12) Amidite **15** has successfully been used for synthesis of α-L-xylo-LNA[5] on an automated DNA synthesizer using the same conditions described for synthesis of α-L-LNA.[4a]

Experimental Section

General Procedure. Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. Column chromatography was carried out on glass columns using Silica gel 60 (0.040−0.063 mm). After drying organic phases using Na_2SO_4, filtration was performed. Petroleum ether of distillation range 60−80 °C was used. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard for ^1H (300 or 400 MHz) and ^{13}C (75.5 or 100.6 MHz) and relative to 85% H_3PO_4 (121.5 MHz) as external standard for ^{31}P. Assignments of NMR spectra when given are based on 2D spectra and follow standard nucleoside style; i.e. the carbon atom next to the nucleobase is assigned C-1′ etc. (C-5″ designates the carbon atom in the 4′-*C*-branch). However, compound names in the Experimental Section for the bicyclic compounds are given according to the von Baeyer nomenclature. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode. Microanalyses were performed at The Microanalytical Laboratory, Department of Chemistry, University of Copenhagen.

1-(3-*O*-Benzyl-4-*C*-(4,4′-dimethoxytrityloxymethyl)-β-D-xylofuranosyl)thymine (2). To a solution of 1-(3-*O*-benzyl-4-*C*-hydroxymethyl-β-D-xylofuranosyl)thymine (**1**)[6] (5.38 g, 14.2 mmol) in anhydrous tetrahydrofuran (THF) (400 mL) was added $AgNO_3$ (2.66 g, 15.7 mmol) followed by anhydrous pyridine (5.7 mL) and 4,4′-dimethoxytrityl chloride ((DMT)-Cl, 5.30 g, 15.6 mmol). The mixture was stirred in the dark for 18 h at room temperature whereupon the reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$, and the resulting mixture was extracted with CH_2Cl_2. The combined organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene and was purified by silica gel column chromatography using CH_2Cl_2/MeOH/pyridine (99:0.5:0.5 (v/v/v)) as eluent affording nucleoside **2** (3.13 g, 31%) as a white foam after evaporation of the solvents and coevaporation with toluene. ^1H NMR ($(CD_3)_2SO$) δ 11.34 (s, 1H, NH), 7.86 (s, 1H, 6-H), 6.85−7.40 (m, 18H, m, DMT, Bn), 5.93 (d, 1H, *J* 7.3 Hz, 1′-H), 5.80 (d, 1H, *J* 5.5 Hz, 2′-OH), 4.82 (t, 1H, *J* 5.3 Hz, 5′-OH), 4.63 (d, 1H, *J* 12.0 Hz, Bn), 4.42−4.46 (m, 2H, Bn, 2′-H), 4.14 (d, 1H, *J* 7.3 Hz, 3′-H), 3.72 (s, 6H, DMT), 3.65−3.69 (m, 1H, 5′-H), 3.50−3.47 (m, 1H, 5′-H), 3.09 (d, 1H, *J* 9.7 Hz, 5″-H), 2.98 (d, 1H, *J* 9.7 Hz, 5″-H), 1.78 (s, 3H, CH_3). ^{13}C NMR ($(CD_3)_2SO$) δ 164.1 (C-4), 158.4, 145.1, 138.5, 137.0, 135.9, 135.7, 130.1, 130.1, 129.2, 128.5, 128.5, 128.2, 128.1, 127.7, 127.6, 127.0, 125.7, 113.5 (DMT, Bn, C-6), 151.4 (C-2), 110.1 (C-5), 85.8, 85.2, 84.6, 83.5 (C-1′, C-3′, C-4′, DMT), 76.8 (C-2′), 72.3 (Bn), 65.2 (C-5″), 62.1 (C-5′), 55.4 (DMT), 12.6 (CH_3).

1-(3-*O*-Benzyl-4-*C*-(4,4′-dimethoxytrityloxymethyl)-2,5-di-*O*-(*p*-toluenesulfonyl)-*β*-D-xylofuranosyl)thymine (3). To a solution of nucleoside **2** (2.79 g, 3.9 mmol) in anhydrous pyridine (10 mL) was added *p*-toluenesulfonyl chloride (TsCl, 6.50 g, 34 mmol) and a catalytic amount of DMAP. The mixture was stirred in the dark for 24 h at room temperature whereupon the reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ (25 mL). The resulting mixture was extracted with CH_2Cl_2, and the combined organic phase was washed successively with saturated aqueous solutions of $NaHCO_3$ and sodium chloride, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/pyridine (99:0.5:0.5 (v/v/v)) as eluent, yielding nucleoside **3** (2.40 g, 63%) as a yellowish foam after evaporation of the solvents. FAB-MS *m*/*z* 989 $[M + H]^+$, 1011.4 $[M + Na]^+$; ^1H NMR ($(CD_3)_2SO$) δ 11.29 (s, 1H, NH), 8.58 (s, 1H, 6-H), 6.84−7.66 (m, 26H, DMT, Bn, Ts), 6.10 (d, 1H, *J* 7.7 Hz, 1′-H), 5.27 (t, 1H, *J* 7.2 Hz, 2′-H), 4.44 (d, 1H, *J* 6.8 Hz, 3′-H), 4.39 (d, 1H, *J* 11.4 Hz, Bn), 4.24 (d, 1H, *J* 11.5 Hz, Bn), 4.18 (d, 1H, *J* 10.4 Hz, 5′-H), 4.13 (d, 1H, *J* 10.3 Hz, 5′-H), 3.73 (s, 3H, DMT), 3.73 (s, 3H, DMT), 3.14 (d, 1H, *J* 10.3 Hz, 5″-H), 3.07 (d, 1H, *J* 10.3 Hz, 5″-H), 2.35 (s, 3H, Ts), 2.34 (s, 3H, Ts), 1.72 (s, 3H, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.2 (C-4), 158.2, 145.9, 145.1, 144.3, 136.8, 135.0, 134.9, 134.8, 131.8, 131.6, 130.2, 130.0, 129.7, 128.2, 127.9, 127.8, 127.6, 127.5, 127.5, 127.4, 126.8, 113.3 (DMT, C-6, 2 × Ts, Bn), 150.2 (C-2), 110.8 (C-5), 95.0, 86.2 (DMT, C-4′), 82.2, 81.9 (C-1′, C-2′), 81.2 (C-3′), 72.9 (Bn), 79 (C-5″), 64 (C-5′), 55.1 (DMT), 21.2, 21.2 (2 × Ts), 12.0 (CH_3).

(1*S*,3*R*,4*S*,7*R*)-7-Benzyloxy-1-(4,4′-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (4). To a solution of nucleoside **3** (3.87 g, 3.92 mmol) in a mixture of EtOH and H_2O (100 mL, 1:1 (v/v)) was added a solution of aqueous NaOH (2 M, 8 mL). The mixture was heated under reflux for 24 h and, after cooling to room temperature, extracted with CH_2Cl_2. The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$, dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/pyridine (99:0.5:0.5 (v/v/v)) as eluent, affording nucleoside **4** (2.10 g, 81%) as a white foam after evaporation of the solvents. FAB-MS *m*/*z* 663 $[M + H]^+$; ^1H NMR ($(CD_3)_2SO$) δ 11.40 (s, 1H, NH), 7.62 (s, 1H, 6-H), 6.84−7.43 (m, 18H, DMT, Bn), 6.04 (s, 1H, 1′-H), 4.70 (d, 1H, *J* 11.9 Hz, Bn), 4.64 (s, 1H, 2′-H), 4.60 (d, 1H, *J* 12.1 Hz, Bn), 4.57 (s, 1H, 3′-H), 4.11 (d, 1H, *J* 8.2 Hz, 5′-H), 3.88 (d, 1H, *J* 8.6 Hz, 5′-H), 3.73 (s, 3H, DMT), 3.72 (s, 3H, DMT), 3.36 (d, 1H, *J* 11.0 Hz, 5″-H), 3.26 (d, 1H, *J* 10.8 Hz, 5″-H), 1.84 (s, 3H, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.8 (C-4), 158.2, 158.1, 144.7, 137.7, 135.9, 135.2, 135.1, 129.8, 129.7, 128.3, 127.9, 127.7, 127.7, 127.4, 126.7, 113.35 (DMT, Bn, C-6) 150.3 (C-2), 108.1 (C-5), 88.4, 85.5 (C-4′, DMT), 86.4 (C-1′), 79.5 (C-2′), 76.3 (C-3′), 72.6 (C-5′), 71.2 (Bn), 58.9 (C-5″), 55.1 (DMT), 12.4 (CH_3).

(1*S*,3*R*,4*S*,7*R*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (5) and (1*R*,3*R*,4*S*,7*R*)-7-Hydroxy-1-(hydroxyoxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (6). To a solution of nucleoside **4** (1.09 g, 1.65 mmol) in MeOH (90 mL) was added ammonium formate (0.33 g, 5.29 mmol). A catalytic amount of 10% Pd/C suspended in MeOH (10 mL) was added, and the mixture was heated for 2 h under reflux. After cooling to room temperature, the mixture was evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/pyridine (97.5:2:0.5 (v/v/v)) as eluent yielding nucleoside **5** (0.76 g, 80%) and nucleoside **6** (148 mg, 17%) as white solid materials after evaporation of the solvents. **Nucleoside 5:** FAB-MS *m*/*z* 573 $[M + H]^+$; ^1H NMR ($(CD_3)_2SO$) δ 11.39 (s, 1H, NH), 7.62 (s, 1H, 6-H), 6.89−7.44 (m, 13H, DMT), 5.97 (s, 1H, 1′-H), 5.93 (d, 1H, *J* 4.3 Hz, 3′-OH), 4.44 (d, 1H, *J* 4.3 Hz, 3′-H), 4.23 (s, 1H, 2′-H), 4.13 (d, 1H, *J* 8.4 Hz, 5′-H), 3.92 (d, 1H, *J* 8.4 Hz, 5′-H), 3.74 (s, 6H, DMT), 3.31 (s, 2H, 5″-H), 1.86 (s, 3H, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.9 (C-4), 158.2, 144.8, 135.8, 135.4, 135.3, 129.8, 127.9, 127.7, 126.8, 113.3 (DMT, C-6), 150.4 (C-2), 108.0 (C-5), 89.2, 85.4 (C-4′, DMT), 86.4 (C-1′), 78.9 (C-2′), 72.9 (C-3′), 72.3 (C-5′), 59.9 (C-5″), 55.1 (DMT), 12.5 (CH_3). **Nucleoside 6:** FAB-MS *m*/*z* 271 $[M + H]^+$; ^1H NMR ($(CD_3)_2SO$) δ 11.34 (s, 1H, NH), 7.63 (s, 1H, 6-H), 5.97 (d, 1H, *J* 4.3 Hz, 3′-OH), 5.96 (s, 1H, 1′-H), 4.94 (t, 1H, *J* 5.8 Hz, 5″-OH), 4.27 (d, 1H, *J* 4.1 Hz, 3′-H), 4.20 (s, 1H, 2′-H), 3.94 (d, 1H, *J* 8.4 Hz, 5′-H), 3.92 (d, 1H, *J* 8.4 Hz, 5′-H), 3.73 (d, 2H, *J* 5.6 Hz, 5″-H), 1.93 (s, 3H, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.9 (C-4), 150.4 (C-2), 136.0 (C-6), 107.9 (C-5), 91.0 (C-4′), 86.5 (C-1′), 78.9 (C-2′), 72.5 (C-3′), 72.0 (C-5′), 57.5 (C-5″), 12.3 (CH_3). The enantiomer of **6** has been published earlier.[13]

1-(3-*O*-Benzyl-2,5-di-*O*-methanesulfonyl-4-*C*-(methanesulfonyloxymethyl)-*β*-D-xylofuranosyl)thymine (7). To a solution of nucleoside **1**[6] (1.63 g, 4.31 mmol) in anhydrous pyridine (8 mL) at 0 °C was added drop by drop methanesulfonyl chloride (MsCl, 1.2 mL, 15.5 mmol). The mixture was stirred for 2 h at 0 °C and then at 2 h at room temperature. The reaction mixture was cooled to 0 °C, and H_2O (4 mL) was added followed by a saturated aqueous solution of $NaHCO_3$ (10 mL). Extraction was performed with CH_2Cl_2, and the organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (99:1 (v/v)) as eluent, affording nucleoside **7** (2.41 g, 92%) as a white solid material after evaporation of the solvents. FAB-MS *m*/*z* 613 $[M + H]^+$; ^1H NMR ($CDCl_3$) δ 9.53 (br s, 1H, NH), 7.27−7.40 (m, 6H, Bn, 6-H), 6.16 (d, 1H, *J* 3.7 Hz, 1′-H), 5.30 (t, 1H, *J* 3.6 Hz, 2′-H), 4.76 (d, 1H, *J* 11.4 Hz, Bn), 4.66 (d, 1H, *J* 11.4 Hz, Bn), 4.61 (d, 1H, *J* 11.2 Hz), 4.44 (d, 1H, *J* 3.3 Hz, 3′-H), 4.39 (d, 1H, *J* 11.2), 4.34 (d, 1H, *J* 10.8 Hz), 4.26 (d, 1H, *J* 10.8 Hz), 3.19 (s, 3H, Ms), 3.06 (s, 3H, Ms), 3.02 (s, 3H, Ms), 1.86 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.3 (C-4), 150.6 (C-2), 135.6, 134.6 (C-6, Bn), 128.7, 128.3 (Bn), 112.2 (C-5), 87.9 (C-1′), 85.0 (C-4′), 83.1 (C-2′), 80.9 (C-3′), 73.3 (Bn), 66.6, 66.2 (C-5′, C-5″), 38.6, 37.6, 37.6 (3 × Ms), 12.2 (CH_3).

(1*R*,3*R*,4*S*,7*R*)-1-(Hydroxymethyl)-7-benzyloxy-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (8). To a solution of nucleoside **7** (522 mg, 0.85 mmol) in a mixture of EtOH and H_2O (16 mL, 1:1 (v/v)) was added aqueous NaOH (6 M, 1.5 mL). After heating under reflux for 43 h, the mixture was evaporated to dryness under reduced pressure, the residue was dissolved in CH_2Cl_2 (10 mL), and washing was performed using a saturated aqueous solution of $NaHCO_3$. The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH (97.5:2.5 (v/v)) as eluent affording nucleoside **8** (179 mg, 58%) as a white solid material after evaporation of the solvents. FAB-MS *m*/*z* 361 $[M + H]^+$; ^1H NMR ($(CD_3)_2SO$) δ 11.37 (s, 1H, NH), 7.63 (s, 1H, 6-H), 7.27−7.39 (m, 5H, Bn), 5.87 (s, 1H, 1′-H), 5.07 (br s, 1H, 5″-OH), 4.69 (d, 1H, *J* 11.9 Hz), 4.65 (d, 1H, *J* 11.9 Hz), 4.51 (s, 1H, 2′-H), 4.33 (s, 1H, 3′-H), 4.01 (d, 1H, *J* 8.6 Hz), 3.93 (d, 1H, *J* 8.4 Hz), 3.78 (d, 1H, *J* 13.0 Hz), 3.74 (d, 1H, *J* 12.8 Hz), 1.83 (s, 1H, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.8 (C-4), 150.3 (C-2), 138.0, 135.8 (C-6, Bn), 128.3, 127.7, 127.5 (Bn), 108.0 (C-5), 90.2 (C-4′), 86.5 (C-1′), 79.3 (C-3′), 76.5 (C-2′), 72.5, 71.2, 57.2 (Bn, C-5′, C-5″), 12.3 (CH_3).

Alternative Preparation of 4. Nucleoside **8** (179 mg, 0.5. mmol) was coevaporated with anhydrous pyridine (2 × 20 mL), and the residue was dissolved in anhydrous pyridine (20 mL). (DMT)Cl (409 mg, 1,21 mmol) was added, and the reaction mixture was stirred for 24 h at room temperature. A saturated aqueous solution of $NaHCO_3$ (45 mL) was added, and the resulting mixture was extracted with CH_2Cl_2. The combined organic phase was dried ($MgSO_4$) and evaporated to dryness under reduced pressure followed by coevaporation with toluene twice. The residue was purified by silica gel column chromatography using CH_2Cl_2/MeOH/pyridine (2% MeOH, 0.5% pyridine (v/v/v)) as eluent to afford nucleoside **4** (308 mg, 93%) as a yellowish solid material. All analytical data were identical to those reported above for **4**.

Alternative Preparation of 6. To a stirred solution of nucleoside **8** (179 mg, 0.50 mmol) in EtOH (7 mL) at room

(13) Nielsen, P.; Wengel, J. *Chem. Commun.* **1998**, 2645.

temperature was added 20% $Pd(OH)_2/C$ (50 mg). The mixture was degassed and subjected to an atmosphere of hydrogen. After 24 h at room temperature, the mixture was filtered through Celite. The Celite was washed with MeOH, and the combined filtrate was evaporated to dryness under reduced pressure to give nucleoside **6** (132 mg, 98%) as a white solid material. All analytical data were identical to those reported above for **6**.

Alternative Preparation of 5. Nucleoside **6** (132 mg, 0.49 mmol) was coevaporated with anhydrous pyridine (2 × 3 mL), and the residue was dissolved in anhydrous pyridine (5 mL). (DMT)Cl (331 mg, 0.98 mmol) was added, and the mixture was stirred at room temperature for 22 h. The mixture was poured into a mixture of petroleum ether/CH_2Cl_2/H_2O (30 mL, 1:1:1 (v/v)). The organic phase was separated and washed with a saturated aqueous solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 0−1% MeOH (v/v)) to give nucleoside **5** as a white foam (273 mg, 98%). All analytical data were identical to those reported above for **5**.

1-(3-*O*-Benzyl-2,5-di-*O*-methanesulfonyl-4-*C*-(methanesulfonyloxymethyl)-*β*-D-ribofuranosyl)thymine (10). Compound **9**[11] (497 mg, 0.93 mmol) was coevaporated with anhydrous pyridine (2 × 2.5 mL), and the residue was dissolved in anhydrous pyridine (5 mL). To this stirred solution at 0 °C was added MsCl (0.14 mL, 1.86 mmol), and the mixture was then stirred first at 0 °C for 10 min and then at room temperature for 5 h. The mixture was cooled to 0 °C, and H_2O (10 mL) was added. The mixture was extracted with CH_2Cl_2, and the combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (2% MeOH in CH_2Cl_2 (v/v)) to give tri-*O*-mesylate **10** as a white solid material (550 mg, 97%). ^{1}H NMR ($CDCl_3$) δ 9.66 (s, 1H, NH), 7.17−7.38 (m, 6H, 6-H, Bn), 5.80 (d, 1H, *J* 2.3 Hz, 1′-H), 5.57 (dd, 1H, *J* 6.4 and 2.3 Hz, 2′-H), 4.75 (d, 1H, *J* 10.8 Hz), 4.73 (d, 1H, *J* 6.4 Hz, 3′-H), 4.60 (d, 1H, *J* 11.1 Hz), 4.54 (d, 1H, *J* 11.7 Hz), 4.41 (d, 1H, *J* 10.8 Hz), 4.35 (d, 1H, *J* 10.8 Hz), 4.33 (d, 1H, *J* 11.4 Hz), 3.13 (s, 3H, Ms), 3.01 (s, 3H, Ms), 2.98 (s, 3H, Ms), 1.91 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.7 (C-4), 150.3 (C-2), 137.9, 136.2 (C-6, Bn), 128.6, 128.5, 128.4 (Bn), 111.7 (C-5), 93.3 (C-1′), 84.2 (C-4′), 77.6, 76.8, 74.1, 68.1, 67.5 (Bn, C-2′, C-3′, C-5′, C-5″), 38.5, 37.5, 37.4 (3 × Ms), 12.1 (CH_3). Anal. Calcd for $C_{21}H_{28}N_2O_{13}S_3$: C, 41.2; H, 4.6; N, 4.6. Found: C, 40.7; H, 4.3; N, 4.5.

(1*S*,3*R*,4*S*,7*S*)-7-Benzyloxy-1-(methanesulfonyloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (11) and (1*R*,3*R*,4*S*,7*S*)-7-Benzyloxy-1-(hydroxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (12). Compound **10** (1.63 g, 2.66 mmol) was dissolved in a mixture of 96% aqueous EtOH and H_2O (1:1 (v/v), 60 mL). A 2 M amount of aqueous NaOH (10 mL, 20 mmol) was added, and the mixture was heated at 85 °C for 40 h. Analytical TLC revealed the formation of two major products both with lower mobility than **10**. The mixture was evaporated to half volume under reduced pressure, and the resulting mixture was extracted with CH_2Cl_2. The combined organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (0−1% MeOH in CH_2Cl_2 (v/v)) to give nucleosides **11** (408 mg, 35%) and **12** (304 mg, 32%) as white solid materials. **Conversion of 11 to 12.** Compound **11** (138 mg, 0.31 mmol) was dissolved in EtOH (24 mL), and KOH (177 mg, 3.10 mmol) was added. The mixture was heated at 60 °C for 12 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (0−1% MeOH in CH_2Cl_2 (v/v)) to give nucleoside **12** as a white solid material (87 mg, 79%). **Nucleoside 11:** FAB-MS *m*/*z* 439 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.95 (s, 1H), 7.28−7.54 (m, 6H), 6.27 (s, 1H), 4.71 (d, 1H, *J* 11.7 Hz), 4.64 (d, 1H, *J* 12.0 Hz), 4.57 (d, 1H, *J* 11.4 Hz), 4.49 (d, 1H, *J* 11.7 Hz), 4.26 (s, 1H), 4.10 (d, 1H, *J* 9.1 Hz), 4.05 (d, 1H, *J* 8.8 Hz), 3.05 (s, 3H), 1.94 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.7, 150.2, 136.2, 135.5, 128.7, 128.5, 128.0, 109.9, 90.0, 85.9, 81.7, 75.9, 73.1, 65.1, 37.6, 12.6. **Nucleoside 12:** FAB-MS *m*/*z* 361 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.49 (s, 1H, NH), 7.52 (d, 1H, *J* 1.3 Hz, 6-H), 7.26−7.39 (m, 5H, Bn), 6.24 (s, 1H, 1′-H), 4.71 (d, 1H, *J* 11.9 Hz), 4.65 (d, 1H, *J* 12.1 Hz), 4.47 (dd, 1H, *J* 2.4 Hz, *J* 0.9 Hz, 2′-H), 4.22 (d, 1H, *J* 2.4 Hz, 3′-H), 4.05 (m, 3H, 2 × 5″-H, 5′-H[a]), 3.91 (dd, 1H, *J* 12.3 and *J* 8.8 Hz, 5′-H[b]), 2.83 (t, 1H, *J* 6.6 Hz, 5′-OH), 1.92 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.2 (C-4), 150.5 (C-2), 136.7, 136.0 (C-6, Bn), 128.7, 128.4, 128.0 (Bn), 109.6 (C-5), 89.9 (C-1′), 88.7 (C-4′), 81.9 (C-3′), 76.1 (C-2′), 73.9 (C-5″), 73.0 (Bn), 59.0 (C-5′), 12.7 (CH_3).

(1*R*,3*R*,4*S*,7*S*)-7-Hydroxy-1-(hydroxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (13). Compound **12** (87 mg, 0.24 mmol) was dissolved in EtOH (2.5 mL) and 20% $Pd(OH)_2/C$ (25 mg) was added. The mixture was degassed several times with N_2 and placed under an atmosphere of hydrogen. After stirring for 14 h at room temperature, the mixture was filtered through Celite and the solvent was evaporated under reduced pressure to give compound **13** as a white solid material (61 mg, 93%). FAB-MS *m*/*z* 271 $[M + H]^+$, 289 $[M + 19]$; ^{1}H NMR ($(CD_3)_2SO$) δ 11.34 (s, 1H, NH), 7.64 (d, 1H, *J* 1.1 Hz, 6-H), 6.10 (s, 1H, 1′-H), 5.91 (d, 1H, *J* 3.8 Hz, 3′-OH), 4.94 (t, 1H, *J* 5.5 Hz, 5′-OH), 4.32 (m, 1H, 3′-H), 4.18 (dd, 1H, *J* 0.7 Hz, *J* 2.6 Hz, 2′-H), 4.04 (s, 2H, 5″-H), 3.72 (d, 2H, *J* 4.4 Hz, 5′-H), 1.83 (d, 3H, *J* 0.9 Hz, CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.9 (C-4), 150.4 (C-2), 136.5 (C-6), 108.0 (C-5), 89.2, 89.1 (C-1′, C-4′), 77.3 (C-2′), 74.7 (C-3′), 73.6 (C-5″), 57.3 (C-5′), 12.3 (CH_3). Anal. Calcd For $C_{11}H_{14}N_2O_6 \cdot 0.2H_2O$: C, 48.3; H, 5.3; N, 10.2. Found: C, 48.2; H, 5.3; N, 9.8.

(1*S*,3*R*,4*S*,7*S*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (14). Compound **13** (280 mg, 1.04 mmol) was coevaporated with anhydrous pyridine (5 × 6 mL), and the residue was dissolved in anhydrous pyridine (8 mL). (DMT)Cl (678 mg, 2.04 mmol) was added, and the mixture was stirred at room temperature for 8 h. The mixture was poured into a mixture of petroleum ether/CH_2Cl_2/H_2O (30 mL, 1:1:1 (v/v)). The organic phase was separated and washed with a saturated aqueous solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH/pyridine, 0−2% MeOH, 0.5% pyridine (v/v/v)) to give nucleoside **14** as a white solid material (563 mg, 95%). FAB-MS *m*/*z* 573 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.62 (s, 1H), 7.57 (d, 1H, *J* 1.2 Hz), 7.19−7.43 (9H, m), 6.81−6.86 (4H, m), 6.31 (s, 1H), 4.52 (m, 2H), 4.33 (1H, br s), 4.08 (d, 1H, *J* 9.1 Hz), 3.98 (d, 1H, *J* 8.8 Hz), 3.78 (s, 6H), 3.57 (d, 1H, *J* 10.3 Hz), 3.51 (d, 1H, *J* 10.3 Hz), 1.94 (d, 3H, *J* 1.2 Hz); ^{13}C NMR ($CDCl_3$) δ 164.0, 158.5, 150.4, 149.3, 144.1, 136.1, 136.0, 135.0, 129.9, 127.8, 126.9, 123.8, 109.4, 90.1, 88.2, 86.6, 77.8, 76.1, 74.2, 59.7, 12.7.

(1*S*,3*R*,4*S*,7*S*)-7-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-1-(4,4′-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (15). Compound **14** (200 mg, 0.35 mmol) was coevaporated with anhydrous acetonitrile (3 × 2 mL) and dissolved in anhydrous CH_2Cl_2 (4 mL). The solution was stirred under N_2 and cooled to 0 °C. *N*,*N*-Diisopropylethylamine (DIPEA, 0.18 mL, 1.05 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.17 mL, 0.51 mmol) were added. The mixture was heated to room temperature, and stirring was continued for 12 h. After 2, 4, and 6 h, respectively, additional DIPEA (0.18 mL, 1.05 mmol) and 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.17 mL, 0.51 mmol) were added. After an additional 2 h, MeOH (0.05 mL) was added, and the mixture was diluted with CH_2Cl_2 (30 mL) and washed with a saturated aqueous solution of $NaHCO_3$ and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (EtOAc/*n*-hexane/NEt_3, 45:45:10 (v/v/v)) to give a white foam after evaporation under reduced pressure. This residue was dissolved in anhydrous toluene (0.5 mL), and the product was precipitated by addition drop by drop of this solution to petroleum ether (50 mL, cooled to −30 °C) under vigorous stirring. The precipitate was collected by filtration and dried to give compound **15** as a white solid

material (161 mg, 60%). FAB-MS *m*/*z* 773 $[M + H]^+$; ^{31}P NMR ($(CD_3)_2SO$) δ 150.8, 150.5.

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Supporting Information Available: Figures showing ^{13}C NMR spectra for compounds **2**-**7** and **10**-**14** and the ^{31}P NMR spectrum for compound **15**. This information is available free of charge via the Internet at http://pubs.acs.org.

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