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α-L-*ribo*-Configured Locked Nucleic Acid (α-L-LNA): Synthesis and Properties

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Abstract: The syntheses of monomeric nucleosides and 3′-*O*-phosphoramidite building blocks en route to α-L-*ribo*-configured locked nucleic acids (α-L-LNA), composed entirely of α-L-LNA monomers (α-L-*ribo* configuration) or of a mixture of α-L-LNA and DNA monomers (*β*-D-*ribo* configuration), are described and the α-L-LNA oligomers are studied. Bicyclic 5-methylcytosin-1-yl and adenin-9-yl nucleoside derivatives have been prepared and the phosphoramidite approach has been used for the automated oligomerization leading to α-L-LNA oligomers. Binding studies revealed very efficient recognition of single-stranded DNA and RNA target oligonucleotide strands. Thus, stereoirregular α-L-LNA 11-mers containing a mixture of α-L-LNA monomers and DNA monomers ("mix-mer α-L-LNA") were shown to display ΔT_m values of +1 to +3 °C per modification toward DNA and +4 to +5 °C toward RNA when compared with the corresponding unmodified DNA•DNA and DNA•RNA reference duplexes. The corresponding ΔT_m values per modification for the stereoregular fully modified α-L-LNA were determined to be +4 °C (against DNA) and +5 °C (against RNA). 11-Mer α-L-LNAs (mix-mer α- L-LNA or fully modified α- L-LNA) were shown in vitro to be significantly stabilized toward 3′-exonucleolytic degradation. A duplex formed between RNA and either mix-mer α-L-LNA or fully modified α-L-LNA induced in vitro *Escherichia coli* RNase H-mediated cleavage, albeit very slow, of the RNA targets at high enzyme concentrations.

Introduction

The prospect of preparing therapeutically active analogues of natural nucleic acids has stimulated much interest in the synthesis of effective antisense oligonucleotides (AONs) during the past decade. Some of the requirements for an AON include efficient automated synthesis, good aqueous solubility, resistance toward enzymatic degradation, and high binding affinity toward nucleic acid targets while maintaining the fidelity of recognition.[1] The unprecedented thermal stability of duplexes involving LNA (locked nucleic acid, *β*-D-*ribo* isomer, Figure 1)[2,3] has inspired us to investigate the properties of the stereoisomers of LNA. Recently, synthesis of the stereoisomeric analogues termed *xylo*-LNA (*β*-D-*xylo* isomer),[3,4a−c] α-L-xylo-LNA (α-L-*xylo* isomer),[3,4c,5] α-L-LNA (α-L-*ribo* isomer, Figure 1),[3,4a−d,5,6] and α-LNA (α-D-*ribo* isomer)[4e] and their thermal stability toward complementary nucleic acid targets have been reported. These

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(3) 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 monomers) as the positioning of the N1, O2′, O3′, and C5′ atoms are equivalent to those found in RNA. Analogously, *xylo*-LNA, α-L-*xylo*-LNA, and α-L-LNA have been defined as oligonucleotides containing one or more 2′-*O*,4′-*C*-methylene-*β*-D-xylofuranosyl nucleotide monomer(s), 2′-*O*,4′-*C*-methylene-α-L-xylofuranosyl nucleotide monomer(s), and 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl nucleotide monomer(s), respectively. The term "mix-mer α-L-LNA" is used herein for an α-L-LNA containing a mixture of α-L-LNA monomers and DNA monomers, and the term "mix-mer LNA" is used for an LNA containing a mixture of LNA monomers and DNA monomers.

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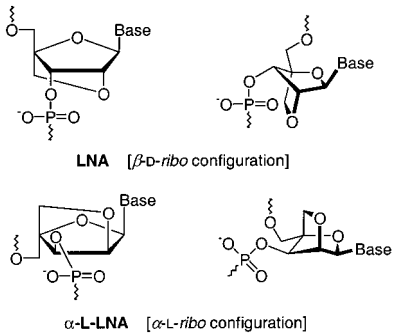


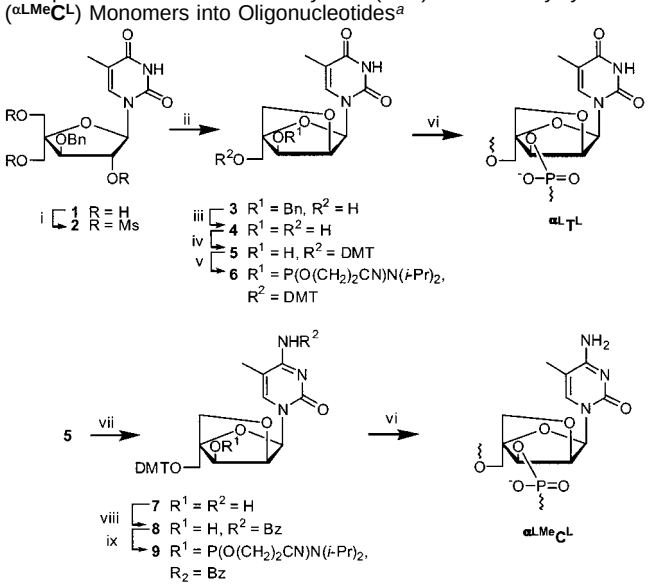
Figure 1. Structures of the nucleotide monomers of LNA and α-L-LNA (left) and sketches of their locked *N*-type[7] (C3′-*endo*/^3E) furanose conformations (right).

preceding studies revealed very efficient recognition of single-stranded DNA and RNA by both LNA and α-L-LNA. Due to the fact that the furanose rings of both LNA and α-L-LNA monomers are part of a dioxabicyclo[2.2.1]heptane skeleton, they are both efficiently locked in an *N*-type (C3′-*endo*/^3E) conformation (Figure 1).[7] So far, the properties of α-L-LNA containing the thymine and the adenine α-L-LNA monomers have been studied, i.e., homothymine oligomers[4a−c] and 9-mer α-L-LNAs containing a mixture of α-L-LNA monomers (three thymine[4c] or three adenine α-L-LNA monomers[4d]) and DNA monomers ("mix-mer α-L-LNAs").[3,4c,4d] On the basis of these preliminary studies, the surprising indication is that the helical thermostabilities of α-L-LNA·DNA and α-L-LNA·RNA duplexes approach those of the corresponding LNA·DNA and LNA·RNA duplexes.[2,4a−4d]

One mode of action of AONs involves recruitment of the enzyme RNase H for the degradation of the targeted mRNA, but the exact recognition elements of this substrate-specific endogenous enzyme are not known at present time.[8] When hybridized to complementary RNA, LNA has been recognized as an RNA mimic that drives the duplex to adopt an A-type conformation.[2d,9] This feature is disadvantageous in terms of RNase H recognition because this enzyme recognizes DNA·RNA duplexes intermediary between the DNA B-type and the RNA A-type conformations.[10] NMR spectroscopic studies of α-L-LNA·RNA duplexes and molecular dynamics (MD) simulations of fully modified α-L-LNA·RNA duplexes have shown the overall duplex geometry to be very similar to the corresponding unmodified DNA·RNA hybrid,[6,11] pointing to α-L-LNA being most adequately described as a DNA mimic.

To allow a comprehensive evaluaton of the properties of α-L-LNA and its potential as an AON, we report herein (a) the syntheses of the bicyclic 5-methylcytosin-1-yl and adenin-9-yl α-L-LNA nucleosides and 3′-*O*-phosphoramidite building blocks thereof, (b) binding studies of 11-mer fully modified and 11-mer mix-mer α-L-LNA toward complementary DNA and RNA targets, (c) the behavior of α-L-LNA toward 3′-exonucleolytic degradation in vitro, and (d) the results of *Escherichia coli* RNase H-mediated cleavage of the RNA strand of α-L-LNA·RNA duplexes in vitro.

Scheme 1. Synthesis of the α-L-LNA Thymine[4a,5] and 5-Methylcytosine Phosphoramidite Derivatives **6** and **9** Suitable for Incorporation of the α-L-LNA Thymine ($^{\alpha L}\mathbf{T}^{L}$) and 5-Methylcytosine ($^{\alpha LMe}\mathbf{C}^{L}$) Monomers into Oligonucleotides[a]



[a] Reagents, conditions and yields: (i−v) See refs 4a and 5; (i) MsCl, pyridine, rt (92%); (ii) 6 M NaOH, EtOH, H_2O, reflux (58%); (iii) H_2/(20% $Pd(OH)_2$/C)/EtOH, rt (98%); (iv) DMTCl, pyridine, rt (98%); (v) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (51%; optimized relative to ref 4a); (vi) DNA synthesizer; (vii) (a) TMSCl, CH_2Cl_2, pyridine, rt; (b) Tf_2O, rt; (c) saturated aqueous NH_3 in MeOH, rt (80%); (viii) (a) TMSCl, pyridine, rt; (b) *N*-benzoyl-1*H*-tetrazole, rt (80%); (ix) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (45%). DMTCl = 4,4′-dimethoxytrityl chloride.

Results

Synthesis of α-L-LNA Pyrimidine Nucleosides. Conversion of the known 4′-*C*-hydroxymethyl nucleoside **1** obtained from 1,2:5,6-di-*O*-isopropylidine-α-D-glucofuranose as earlier described,[12] into α-L-LNA pyrimidine phosphoramidite building blocks was performed as depicted in Scheme 1. Transformation of nucleoside **1** into the bicyclic thymine α-L-LNA nucleoside **4** and the corresponding phosphoramidite monomer **6** has been described earlier.[4a,5] The key step was a cascade reaction of tri-*O*-mesyl nucleoside **2** involving C2′ epimerization, hydrolysis of the resulting anhydronucleoside intermediate, cyclization, and removal of the mesyl group at C5′ to give the bicyclic nucleoside **3** in 58% yield.[5] The yield of this cascade reaction was later improved to 89% by use of the corresponding tri-*O*-tosyl derivative of nucleoside **2** as reactant instead (unpublished results).

Synthesis of the 5-methylcytosine α-L-LNA amidite **9** was achieved starting from the thymine nucleoside derivative **5**[5] (Scheme 1). Often, the frequently used triazolation strategy[13] for conversion of thymine into 5-methylcytosine nucleosides

(7) Despite the apparent differences in the furanose conformations between an LNA monomer and an α-L-LNA monomer (Figure 1), also the furanose conformation of an α-L-LNA monomer is of the *N*-type (C3′-*endo*, ^3E) because of its L-configuration. For further information about the conformations of nucleotides, see *Eur. J. Biochem.* **1983**, *131*, 9 (Abbreviations and Symbols for the Description of Conformations of Polynucleotide Chains, IUPAC−IUB Joint Commission on Biochemical Nomenclature).

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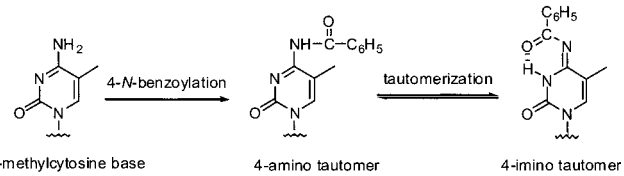

Figure 2. Nucleobase tautomerization of 4-*N*-benzoyl-5-methyl-2′-deoxycytidine[18] and derivative **8**.

gives only moderate overall yields. An alternative method described by Reese and co-workers[14] involving trimethylsilylation of the hydroxy groups of nucleoside **4** followed by activation of the nucleobase with trifluoroacetic anhydride and subsequent treatment with 4-nitrophenol failed in our hands. Instead, the conversion was achieved by a slightly different procedure described by Herdewijn and Van Aerschot.[15] In a transient protection strategy,[16] the 3′-hydroxy group of nucleoside **5** was initially trimethylsilylated in a mixture of pyridine and dichloromethane. The nucleobase was subsequently activated by dropwise addition of 3 equiv of trifluoromethanesulfonic anhydride, furnishing the 5-methylcytosin-1-yl nucleoside **7** in 81% yield after treatment with saturated methanolic ammonia. Nucleoside **7** was transiently protected by trimethylsilylation followed by benzoylation of the nucleobase with *N*-benzoyl-1*H*-tetrazole[17] and subsequent desilylation, furnishing the protected nucleoside **8** in 80% yield. Eventually, standard phosphitylation with 2-cyanoethyl (*N*,*N*-diisopropyl)phosphoramidochloridite and (*N*,*N*-diisopropyl)ethylamine in dichloromethane afforded the desired 4-*N*-benzoyl-5-methylcytosine phosphoramidite building block **9** in 45% yield. Because of the rather efficient synthesis of the thymine nucleoside **5**, we chose the strategy described above for the synthesis of 5-methylcytosine derivative **8** instead of the alternative route involving coupling between an appropriately protected furanose, e.g., derivative **10** or **26** (vide infra), and silylated 5-methylcytosine (or cytosine). Generally, the ^{13}C NMR chemical shift values of the nucleobase change only slightly upon 4-N-acylation of cytosine nucleosides. However, this is not always the case upon 4-N-acylation of 5-methylcytosine nucleosides.[18] Thus, the observed significant downfield shift of the signal for C5 and upfield shifts of the signals for C2, C4, and C6 of nucleoside **8** compared with the values for the nucleoside **7** with an unprotected 5-methylcytosine moiety can be explained by a tautomeric change of the nucleobase (Figure 2). Previously, the nucleobase of 4-*N*-benzoyl-5-methyl-2′-deoxycytidine has been shown to adopt an imino tautomeric form, and the similarity between the ^{13}C NMR shift values reported for this nucleoside[18,19] and the corresponding values for the nucleobase of nucleoside **8** also indicate the imino tautomeric form to be predominant in this case.

Synthesis of α-L-LNA Purine Nucleosides. In general, a reaction sequence similar to the one used above for the synthesis of pyrimidine α-L-LNA nucleosides cannot be applied in the synthesis of the corresponding purine nucleosides because of the inability of the latter to form C2′-anhydronucleoside intermediates. Alternatively, the known tetra-*O*-acyl-L-*threo*-pentofuranose **10** (Scheme 2)[12,20] was used as a starting material in the synthesis of the adenine phosphoramidite building block **23**.[4d] Coupling between furanose **10** and 6-*N*-benzoyladenine with tin(IV) chloride as the Lewis acid afforded the desired N9-regioisomeric nucleoside **11** in 52% yield as the only regio- and stereoisomer isolated, which can be explained by the presence of the participating 2-*O*-acetyl group of **10** and by the fact that the coupling reaction was performed under thermodynamic control. A similar Vorbrüggen-type[21] coupling reaction with trimethylsilyltriflate as Lewis acid and *N*,*O*-bis(trimethylsilyl)acetamide as silylating agent provided nucleoside **11** in only 41% yield (data not shown). By comparison of the ^{13}C NMR chemical shift values obtained for derivatives **20** and **21** (vide infra) with the published values for N7- and N9-regioisomeric β-D-ribofuranosyladenines,[22] it was possible to assign the synthesized nucleoside derivatives **11**−**23** as the N9-regioisomers. Epimerization at C2′ via intermediates **12** and **13** was performed by a reaction sequence in which the 2′-*O*-acetyl group of nucleoside **11** was first deacylated chemoselectively with half-saturated methanolic ammonia[23] to give the intermediate **12**. Subsequently, the reaction of **12** with trifluoromethanesulfonic anhydride in a mixture of pyridine and dichloromethane afforded intermediate **13**, which was directly treated with potassium acetate and 18-crown-6 to afford the desired C2′-epimeric nucleoside **14** in 84% yield (from **11**). Complete deacylation of nucleoside **14** was accomplished with a mixture of saturated methanolic ammonia and saturated aqueous ammonia to give an intermediate, which directly was subjected to a transient protection protocol[16] (O-trimethylsilylation with chlorotrimethylsilane in pyridine, 6-N-benzoylation with benzoyl chloride, and desilylation) giving the 6-*N*-benzoyl derivative **15** in 91% yield from **14**. Nucleoside **15** was mesylated selectively by reaction with 2.2 equiv of mesyl chloride in pyridine at 0 °C, furnishing derivative **16** in 79% yield without any observed mesylation of the secondary hydroxy group. Cyclization by intramolecular nucleophilic attack of the 2′-hydroxy group with concomitant demesylation and debenzoylation was performed in a mixture of aqueous sodium hydroxide and 1,4-dioxane (reflux, 72 h) in only 13% yield. Instead, nucleoside **16** was treated with sodium hydride in THF to give the bicyclic nucleoside **17**, followed by substitution of the remaining mesyloxy group with acetate by reaction with potassium acetate and 18-crown-6 in dioxane at reflux to afford nucleoside **18** in 87% yield (from **16**). Chemoselective O-deacetylation of **18** in half-saturated methanolic ammonia afforded nucleoside **19** in 83% yield, and debenzylation of this intermediate with ammonium formate and Pd/C gave the desired 6-*N*-benzoyl α-L-LNA adenine nucleoside **20** in 44% yield in addition to the fully deprotected nucleoside **21** in 24% yield. The α-L-*ribo* configuration of nucleoside **20** was confirmed by ^{1}H NOE experiments [mutual NOE effects between H2/H8 of the adenine

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(19) ^{13}C NMR shift values for the heterocyclic base carbons of 4-*N*-benzoyl-5-methyl-2′-deoxycytidine (see ref 18): [$(CD_3)_2SO$] δ 176.9 (CO), 160.2 (C-4), 148.7 (C-2), 139.6 (C-6), 109.9 (C-5), 13.3 (CH_3). No significant change was observed when $CDCl_3$ was used as solvent.
(20) The synthesis of phosphoramidite **23** from furanose **10** (Scheme 2) has been described in a preliminary report; see ref 4d.
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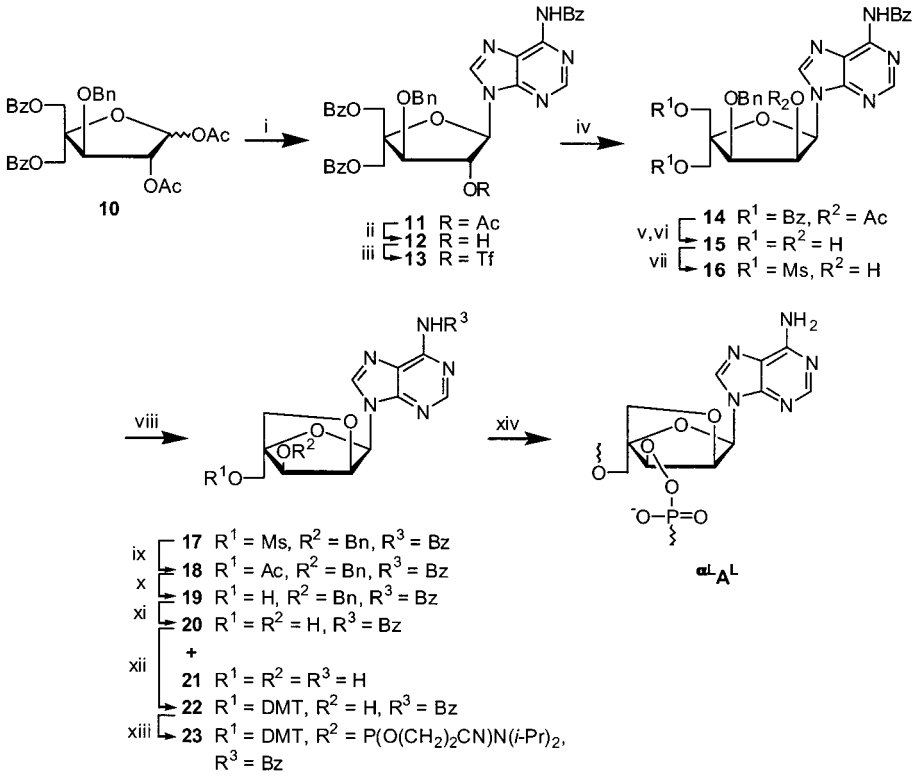
Scheme 2. Synthesis of the α-L-LNA Adenine Phosphoramidite Monomer **23** Suitable for Incorporation of the α-L-LNA Adenine Monomer ($^{\alpha L}\mathbf{A}^{L}$) into Oligonucleotides[a]

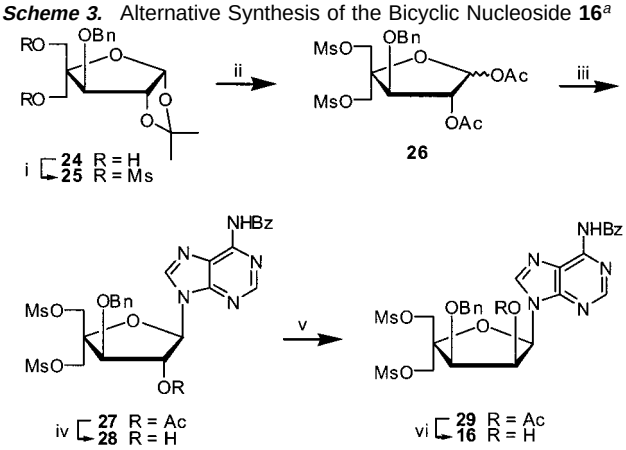


[a] Reagents, conditions and yields: (i) 6-*N*-benzoyladenine, $SnCl_4$, CH_3CN, rt (52%); (ii) half-saturated NH_3 in MeOH, 0 °C; (iii) Tf_2O, pyridine, CH_2Cl_2, −30 to 0 °C; (iv) KOAc, 18-crown-6, toluene, 80 °C (84%, 3 steps); (v) saturated NH_3 in MeOH/32% aq NH_3 (4/1), rt; (vi) (a) TMSCl, pyridine, rt; (b) BzCl, pyridine, rt; (c) 32% aq NH_3/H_2O/MeOH (6/3/4), rt (91%, 2 steps); (vii) MsCl, pyridine, 0 °C (79%); (viii) NaH, THF, rt; (ix) KOAc, 18-crown-6, 1,4-dioxane, 100 °C (87%, 2 steps); (x) half-saturated NH_3 in MeOH, 0 °C (83%); (xi) $HCOONH_4$, 10% Pd/C, MeOH, reflux (**20**, 44%, **21**, 24%); (xii) DMTCl, pyridine, rt (82%); (xiii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (63%); (xiv) DNA synthesizer.

moiety and the protons of the endocyclic methylene group (2% and 8%/2% when H2/H8 and the endocyclic methylene group, respectively, were irradiated) and between H1′ and H2′/H3′ (5%/3% and 11% when H1 and H2′/H3′, respectively, were irradiated)]. To prepare for automated synthesis of α-L-LNA containing the adenine α-L-LNA monomer $^{\alpha L}\mathbf{A}^{L}$, the primary hydroxy group of nucleoside diol **20** was first DMT-protected selectively by reaction with DMTCl in pyridine to give derivative **22** in 82% yield, and second, phosphitylated under standard conditions to furnish the desired phosphoramidite building block **23** in 63% yield (Scheme 2).

In an alternative strategy, the synthesis of intermediate **16** was accomplished with furanose **24**[24] as starting material (Scheme 3). Reaction with excess methanesulfonyl chloride in anhydrous pyridine gave in 79% yield furanose **25**, which was subsequently converted into the 1,2-di-O-acetylated furanose **26** in 63% yield by acetal hydrolysis with 80% trifluoroacetic acid, followed by acetylation with acetic anhydride in pyridine. Coupling between furanose **26** and 6-*N*-benzoyladenine with tin(IV)chloride as Lewis acid afforded stereoselectively the desired N9-regioisomeric nucleoside **27** in 57% yield. By analogy with the synthesis of nucleoside **11**, no other nucleoside products were isolated. Treatment of nucleoside **27** with half-saturated methanolic ammonia gave chemoselectively the O-deacylated intermediate **28** in 79% yield. To prepare for epimerization at C2′, the intermediate **28** was treated with

Scheme 3. Alternative Synthesis of the Bicyclic Nucleoside **16**[a]



[a] Reagents, conditions and yields: (i) MsCl, pyridine, rt (79%); (ii) (a) 80% TFA, rt; (b) Ac_2O, pyridine, rt (63%); (iii) 6-*N*-benzoyladenine, $SnCl_4$, CH_3CN, rt (57%); (iv) half-saturated NH_3 in MeOH, rt (79%); (v) (a) Tf_2O, pyridine, CH_2Cl_2, −30 °C to 0 °C; (b) KOAc, 18-crown-6, toluene, CH_2Cl_2, 50 °C (70%, 2 steps); (vi) half-saturated NH_3 in MeOH, rt (85%).

trifluoromethanesulfonic anhydride in a mixture of pyridine and dichloromethane and subsequently with potassium acetate and 18-crown-6 in a mixture of toluene and dicloromethane to give the desired C2′-epimeric nucleoside **29** in 70% combined yield. In contrast to the unsuccessful O-deacylation of the nucleoside analogue **14**, selective O-deacylation of **29** was achieved with saturated methanolic ammonia at room temperature affording the 6-*N*-benzoyl derivative **16** in 85% yield.[25] The two different routes for synthesis of the adenine monomer differ with respect to both number of steps and overall yields. Thus, the route from

(24) Youssefyeh, R. D.; Verheyden, J. P. H.; Moffat, J. G. *J. Org. Chem.* **1979**, *44*, 1301.

DNAs, α-L-LNAs and LNAs studied:

DNA-1	5'-d(CACACTCAATA)-3'
DNA-2	5'-d(^{Me}CA^{Me}CA^{Me}CT^{Me}CAATA)-3'
DNA-3	5'-d(TCCTG)-d(A)$_6$-d(CGCCG)-3'
α-L-LNA-1	5'-d(C(**$^{\alpha L}$A^{L}**)C(**$^{\alpha L}$A^{L}**)C(**$^{\alpha L}$T^{L}**)C(**$^{\alpha L}$A^{L}**)A(**$^{\alpha L}$T^{L}**)A)-3'
α-L-LNA-2	5'-**$^{\alpha L}$**(^{Me}CA^{Me}CA^{Me}CT^{Me}CAATA)**^{L}**-3'
α-L-LNA-3	5'-d(TCCTG)-(**$^{\alpha L}$A^{L}**)$_6$-d(CGCCG)-3'
LNA-1	5'-d(C(A^{L})C(A^{L})C(T^{L})C(A^{L})A(T^{L})A)-3'
LNA-2	5'-(^{Me}CA^{Me}CA^{Me}CT^{Me}CAATA)^{L}-3'
LNA-3	5'-d(TCCTG)-(A^{L})$_6$-d(CGCCG)-3'

DNA/RNA targets (T) and mis-matched targets (MM):

T1	3'-d(GTGTGAGTTAT)-5'
MM1	3'-d(GTGTAAGTTAT)-5'
MM2	3'-d(GTGTCAGTTAT)-5'
MM3	3'-d(GTGTTAGTTAT)-5'
MM4	3'-d(GTGTGCGTTAT)-5'
MM5	3'-d(GTGTGGGTTAT)-5'
MM6	3'-d(GTGTGTGTTAT)-5'
T2	3'-r(GUGUGAGUUAU)-5'
MM7	3'-r(GUGUCAGUUAU)-5'
MM8	3'-r(GUGUGCGUUAU)-5'

Chimeric 2'-*O*-methyl RNA target:

T3	3'-[2'-OMe(AGGAC)-r(U)$_6$-2'-OMe(GCGGC)]-5'

Figure 3. Various oligonucleotides (α-L-LNAs, LNAs, and unmodified controls and targets) used in this study. α-L-LNA monomers are denoted $^{\alpha L}$**T**^{L}, $^{\alpha LMe}$**C**^{L}, and $^{\alpha L}$**A**^{L}; LNA monomers are denoted **T**^{L}, ^{Me}**C**^{L}, and **A**^{L}; the natural DNA/RNA monomers and 2′-OMe monomers are denoted A, C, ^{Me}C, G, T and U; d(sequence) denotes a DNA strand, r(sequence) an RNA strand, 2′-OMe(sequence) a 2′-*O*-methyl-oligoribonucleotide strand, $^{\alpha L}$(sequence)^{L} a fully modified α-L-LNA strand, and (sequence)^{L} a fully modified LNA strand. Mismatched bases in target sequences **MM1**−**MM8** are underlined.

the starting material **24** via furanose **10** to nucleoside **16** (Scheme 2) proceeded in overall 22% yield (nine steps) whereas the alternative route (Scheme 3) from **24** via **26** to **16** proceeded in an overall yield of only 14% (five steps).

We decided to evaluate α-L-LNA containing thymine, 5-methylcytosine, and adenine monomers ($^{\alpha L}$**T**^{L} , $^{\alpha LMe}$**C**^{L}, and $^{\alpha L}$**A**^{L}, respectively). Having access to two pyrimidine α-L-LNA monomers and one purine α-L-LNA monomer, we envisioned this would allow us to perform the necessary experiments. Preliminary synthetic procedures for preparation of guanine α-L-LNA nucleosides are included in the Supporting Information.

Thermal Denaturation Studies. To test the properties of mix-mer α-L-LNA and fully modified α-L-LNA, we synthesized **α-L-LNA-1**, composed of alternating α-L-LNA (three adenine and two thymidine monomers) and DNA monomers, and the fully modified **α-L-LNA-2** (see Figure 3 for the composition of oligonucleotides). The phosphoramidite approach on an automated DNA synthesizer was used for synthesis of the oligomers (see the Experimental Section for details).

The data illustrating the hybridization properties of **α-L-LNA-1** and **α-L-LNA-2** toward matched and singly mismatched DNA complements (**T1** and **MM1**−**MM6**, respectively) are shown in Table 1. For comparison, the properties of the corresponding DNAs (**DNA-1** and **DNA-2**, containing cytosine and 5-methylcytosine monomers, respectively) and LNAs (**LNA-1** and **LNA-2**) are also shown.

Table 1. Melting Temperatures (T_m Values) toward Matched and Singly Mismatched Complementary DNA[a]

	T_m values/°C						
	T1	**MM1**	**MM2**	**MM3**	**MM4**	**MM5**	**MM6**
DNA-1	**36**	17	10	16	21	27	25
DNA-2	**42**	21	16	22	26	32	28
α-L-LNA-1	**39**	20	11	15	18	33	22
α-L-LNA-2	**65**	40	36	40	46	57	48
LNA-1	**54**	30	27	28	37	45	39
LNA-2	**69**	45	38	42	51	61	50

[a] T_m values for matched sequences are shown in boldface type. T_m values were measured as the maximum of the first derivative of the melting curve (A_{260} vs temperature) recorded in medium salt hybridization buffer (10 mM sodium phosphate, 100 mM sodium chloride, and 0.1 mM EDTA, pH 7.0) with 1.5 μM concentrations of the two strands under the assumption of identical extinction coefficients of modified and unmodified nucleotides.

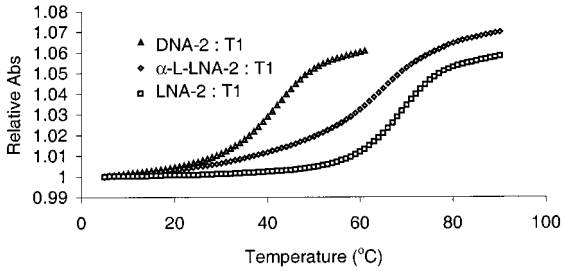


Figure 4. T_m curves for (△) **DNA-2·T1**, (◇) **α-L-LNA-2·T1**, and (□) **LNA-2·T1** duplexes. See the Experimental Section and Table 1 for conditions.

Compared with the reference **DNA-1**, the mix-mer **α-L-LNA-1** increases the T_m value by 3 °C toward the fully matched complementary DNA target **T1**. This increase is surprisingly small compared with the results obtained for 9-mer α-L-LNAs containing three α-L-LNA monomers (either three thymine or three adenine α-L-LNA monomers), for which an increase in the T_m value of 8 °C toward the DNA targets was observed.[4c,4d] In contrast, the LNA mix-mer **LNA-1** induces an increase in the T_m value of 18 °C compared with **DNA-1**. However, the T_m values for the two fully modified 11-mers **α-L-LNA-2** and **LNA-2** when paired with **T1** are comparable and both significantly increased (by 23 and 27 °C, respectively, compared with the value for the DNA reference **DNA-2**). The decreases in thermal stabilities obtained for **α-L-LNA-1** and **α-L-LNA-2** toward singly mismatched DNA targets (**MM1**−**MM6**) are comparable with those obtained for **DNA-1**, **DNA-2**, **LNA-1**, and **LNA-2** (Table 1), although the pairing selectivities of α-L-LNA and LNA in general appear slightly improved compared with that of DNA, thereby confirming the results obtained earlier in other sequence contexts.[2b,4c,4d,26] The normalized melting curves for the duplexes **DNA-2·T1**, **α-L-LNA-2·T1**, and **LNA-2·T1** are depicted in Figure 4.

In Table 2, the hybridization properties of the mix-mer **α-L-LNA-1** and the fully modified **α-L-LNA-2** toward the matched RNA complement **T2** and two singly mismatched RNA complements (**MM7** and **MM8**) are shown and compared with those of the corresponding DNA (**DNA-1** and **DNA-2**) and LNA (**LNA-1** and **LNA-2**) oligomers.

(25) We have, in a preliminary way, attempted yet another alternative way of introducing the bicyclic skeleton of an α-L-LNA adenine nucleoside (data not reported). Thus, the 2′-*O*-mesyl-5′-hydroxy-4′-*C*-hydroxymethyl derivative of nucleoside **12** was refluxed for 94 h with 10 equiv of sodium hydroxide in a 1/1 mixture of water and dioxane. Disappointingly, no ring closure by intramolecular nucleophilic attack from the 5′-hydroxy group on the 2′-position was observed. Attempted ring closure of the corresponding 2′-*O*-trifluoromethanesulfonyl analogue with approximately 6 equiv of sodium hydride in DMF (at room temperature up to 140 °C) for several hours was likewise unsuccessful. In contrast, Robins et al. have reported successful ring closure of the structurally similar nucleoside 9-(3-azido-3-deoxy-2-*O*-mesyl-*β*-D-xylofuranosyl)adenine, affording the corresponding 2′,5′-anhydronucleoside derivative: Robins, M. J.; Hawrelak, S. D.; Kanai, T.; Siefert, J.-M.; Mengel, R. *J. Org. Chem.* **1979**, *44*, 1317.

(26) Ørum, H.; Jacobsen, M. H.; Koch, T.; Vuust, J.; Borre, M. B. *Clin. Chem.* **1999**, *45*, 1898.

Table 2. Melting Temperatures (T_m Values) toward Matched and Singly Mismatched Complementary RNA[a]

	T_m values/°C		
	T2	MM7	MM8
DNA-1	**30**	<10	15
DNA-2	**35**	<10	18
α-L-LNA-1	**49**	25	32
α-L-LNA-2	**75**	50	56
LNA-1	**63**	39	48
LNA-2	**77**	54	61

[a] T_m values for matched sequences are shown in boldface type. See caption below Table 1 for conditions.

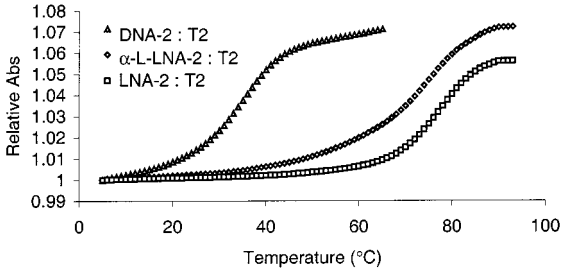


Figure 5. T_m curves for (△) **DNA-2·T2**, (◇) **α-L-LNA-2·T2**, and (□) **LNA-2·T2** duplexes. See the Experimental Section and Table 1 for conditions.

The T_m values for **α-L-LNA-1** and **α-L-LNA-2** toward **T2** are increased by 19 and 40 °C, respectively, compared with the references (**DNA-1** and **DNA-2**, respectively). The corresponding increases for **LNA-1** and **LNA-2** are 33 and 42 °C, respectively. The decreases in thermal stabilities obtained for **α-L-LNA-1** and **α-L-LNA-2** toward the two singly mismatched RNA strands **MM7** and **MM8** are comparable with those obtained for the corresponding DNAs and LNAs, indicating that the hybridization of α-L-LNA with an RNA complement occurs with satisfactory selectivity. The normalized melting curves for the duplexes **DNA-2·T1**, **α-L-LNA-2·T1**, and **LNA-2·T1** are depicted in Figure 5. Taken together, the results obtained clearly demonstrate antiparallel Watson−Crick-type hybridization for α-L-LNA.

The results from the hybridization experiments (Tables 1 and 2) not only confirm the data reported earlier but also reveal important new information. The preferential binding of mix-mer α-L-**LNA** toward RNA has been demonstrated earlier,[4c,4d] although the results obtained herein [compare **α-L-LNA-1·T1** (ΔT_m +3 °C) with **α-L-LNA-1·T2** (ΔT_m +19 °C)] are even more pronounced. It is clear from earlier reported data[2b,4c,4d,26] as well as from the data reported herein that mix-mer LNA displays superior hybridization properties toward especially DNA complements. Importantly, it is shown herein that the binding affinity of fully modified α-L-LNA is comparable to that of fully modified LNA toward both complementary DNA and RNA. A possible reason for the different hybridization behavior of α-L-LNA and LNA as mix-mers can be extracted from results obtained by NMR structural analyses and molecular modeling.[6,9,11] Thus, LNA monomers in mix-mer LNAs have been shown strongly to induce the furanose conformations of neighboring unmodified DNA monomers to shift toward an *N*-type. This leads to preorganization of single-stranded mix-mer LNA and overall A-type conformations of a mix-mer LNA strand in duplexes with both DNA and RNA complements.[9] This conformational tuning toward the duplex-stabilizing A-type conformation[1] explain the very high binding affinities of mix-mer LNAs as also seen in this study (compare data for **DNA-1**, **DNA-2**, **LNA-1**, and **LNA-2**). A similar effect is not seen in mix-mer α-L-LNAs, as the furanose rings of DNA monomers neighboring α-L-LNA monomers retain their *S*-type conformations.[6,11] The reason for the relatively much more efficient hybridization of mix-mer α-L-LNA with RNA (ΔT_m values per modification of α-L-LNA·RNA duplexes compared with DNA·RNA reference duplexes of ca. +5 °C reported earlier[4,11] and ca. +4 °C obtained herein) than with DNA is presently not clear. However, it has been established that α-L-LNA·RNA duplexes are able to adopt overall conformations ranging from an A-form to a form intermediate between the standard A- and B-forms,[11] and differences in single-strand and/or duplex hydration patterns are likely of importance for the preferential RNA-binding of α-L-LNA, as reported earlier for other high-affinity oligonucleotide analogues.[27]

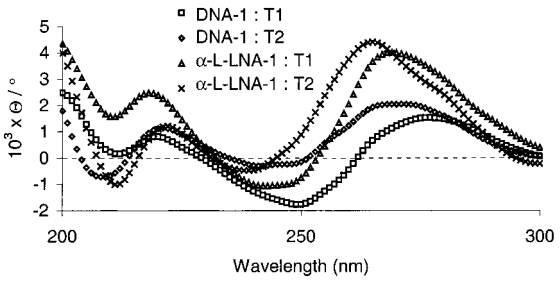


Figure 6. CD spectra of (□) **DNA-1·T1**, (◇) **DNA-1·T2**, (△) **α-L-LNA-1·T1**, and (×) **α-L-LNA-1·T2** duplexes. See Table 1 for hybridization conditions.

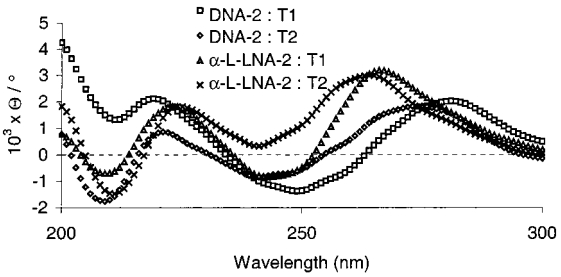


Figure 7. CD spectra of (□) **DNA-2·T1**, (◇) **DNA-2·T2**, (△) **α-L-LNA-2·T1**, and (×) **α-L-LNA-2·T2** duplexes. See Table 1 for hybridization conditions.

CD Spectral Analysis. CD (circular dichroism) spectral analysis allows an estimate of the overall duplex conformation and thus of the general influence of introducing modifications into oligonucleotides. In Figure 6, CD curves of duplexes involving the mix-mer **α-L-LNA-1** are shown compared with those of the unmodified duplexes, whereas Figure 7 depicts the corresponding curves of duplexes involving the fully modified **α-L-LNA-2** and its relevant reference duplexes. Compared with the reference duplexes, a general trend is toward a less intense negative CD band around 240−250 nm and a more intense band around 260−280 nm, the latter being shifted toward shorter wavelengths for the modified duplexes. However, for both **α-L-LNA-1** and **α-L-LNA-2** hybridized to RNA, similarities with the reference DNA·RNA duplexes are demonstrated, indicating the overall duplex conformations as being intermediary between the A- and B-forms. α-L-LNA·DNA duplexes more clearly deviate from the reference DNA·DNA duplexes since the use

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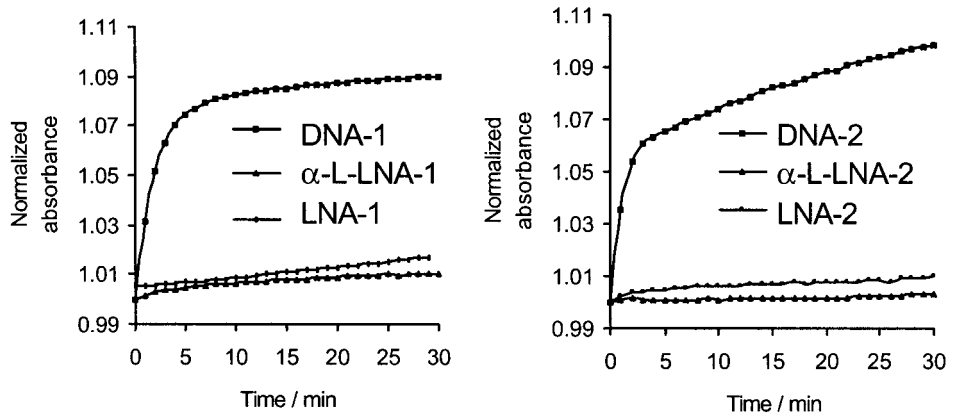

Figure 8. Time course of snake venom phosphodiesterase digestion of α-L-LNA and LNA. See the Experimental Section for details.

of fully modified α-L-LNA seems to induce a shift of the overall conformation of α-L-LNA·DNA duplexes toward an intermediate form approaching that of RNA·DNA duplexes. In summary, the CD spectra shown in Figures 6 and 7 indicate that α-L-LNA oligomers are able to structurally mimic not only DNA but also to some extent RNA.

3′-Exonucleolytic Stability of α-L-LNA in Vitro. To evaluate the stability of α-L-LNA toward 3′-exonucleolytic degradation, we used snake venom phosphodiesterase (SVPDE) in a simple in vitro assay (see the Experimental Section for details).[28,29] During SVPDE digestion of unmodified oligonucleotides, the absorbance (hyperchromicity) at 260 nm increases due to the conversion of the oligonucleotide into monomeric constituents.[30] Figure 8 shows the results for the mix-mers **α-L-LNA-1** and **LNA-1**, the fully modified **α-L-LNA-2** and **LNA-2**, and the corresponding unmodified DNA controls **DNA-1** and **DNA-2**. A rapid increase in absorbance for the unmodified **DNA-1** (and **DNA-2**) follows immediately after addition of SVPDE and declines after less than 5 min. This indicates the expected fast 3′-exonucleolytic degradation of the unmodified DNA.[28,29] It can be seen that both the mix-mers **α-L-LNA-1** and **LNA-1** as well as fully modified **α-L-LNA-2** and **LNA-2** are significantly stabilized toward degradation by SVPDE since no (or only limited) increase in absorbance is observed after 1 h (the time scale depicted is limited to 30 min for clarity). These qualitative experiments indicate that mix-mer α-L-LNA, mix-mer LNA,[31] fully modified α-L-LNA, and fully modified LNA[2a] exhibit significant stabilization toward 3′-exonucleolytic degradation.

In Vitro Cleavage of α-L-LNA/RNA and LNA/RNA Hybrids by *E. coli* RNase H. Recently, a successful method for evaluating the ability of an oligonucleotide analogue to activate RNase H-induced cleavage of its complementary RNA strand was reported that only requires incorporation of the adenine monomer in the oligonucleotide analogue.[32] As poly(dA)/poly(rU) duplexes are poor substrates for *E. coli* RNase H,[32] the method uses a double chimeric approach with six of the desired modified monomers centered in a mixed DNA sequence. To avoid strand cleavage outside the modified region, the RNA complement has 2′-*O*-methylribonucleotide monomers positioned opposite to the unmodified DNA monomers since 2′-*O*-methyl-RNA/DNA hybrids are not substrates of RNase H.[33] *E. coli* RNase H was used because of its easy availability and the fact that its cleavage properties are not very different from those of the mammalian enzyme.[34]

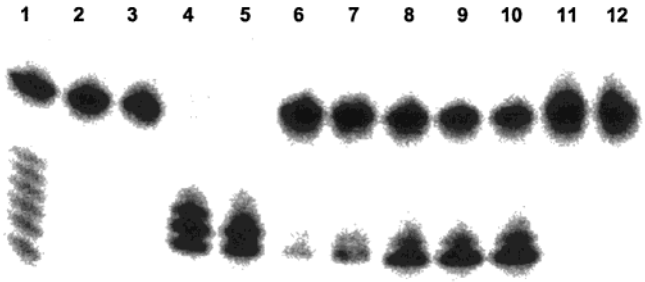

Figure 9. RNase H-mediated hydrolysis of chimeric **T3** upon hybridization with either **DNA-3**, **α-L-LNA-3**, or **LNA-3**. Lane 1, partial alkaline hydrolysis (0.2 M Na_2CO_3, 80 °C, 5 min) of **T3**; lanes 2 and 3, control tubes with the **DNA-3·T3** duplex without RNase H (incubation times 10 min and 21 h); lanes 4 and 5, RNase H activity toward the **DNA-3·T3** duplex (incubation times 10 min and 21 h); lanes 6−10, RNase H activity toward the **α-L-LNA-3·T3** duplex (incubation times 10 min and 1, 4, 8, and 21 h); lanes 11 and 12, RNase H activity toward the **LNA-3·T3** duplex (incubation times 10 min and 21 h).

Initial evaluation of the ability of α-L-LNA to activate RNase H as compared to LNA by the above-mentioned approach is shown in Figure 9. Lane 1 shows partial alkaline hydrolysis of the chimeric RNA target, while lanes 2 and 3 are negative controls of the double chimeric DNA/RNA duplex (**DNA-3·T3**; see Figure 3) after 10 min and 21 h, respectively, without any addition of enzyme. As positive control, the quick degradation of the same DNA/RNA duplex 10 min and 21 h after addition of RNase H, respectively, is shown in lanes 4 and 5. Lanes 6−10 depict cleavage of the **α-L-LNA-3·T3** duplex after 10 min and 1, 4, 8, and 21 h, respectively. As can be seen, considerable cleavage of the RNA strand is only seen after prolonged exposure to the reaction conditions. In contrast, no cleavage at all can be detected for the **LNA-3·T3** duplex, after 10 min or 21 h, as shown in lanes 11 and 12.

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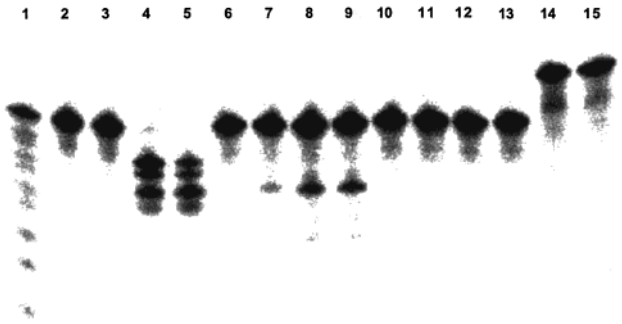

Figure 10. RNase H-mediated hydrolysis of **T2** upon hybridization with either **DNA-1**, **α-L-LNA-1**, **LNA-1**, or **LNA-2**. Lane 1, partial alkaline hydrolysis (0.2 M Na_2CO_3, 80 °C, 7 min) of RNA target; lanes 2 and 3, control tubes with the **DNA-1·T2** duplex without RNase H (incubation times 10 min and 9.5 h); lanes 4 and 5, RNase H activity toward the **DNA-1·T2** duplex (incubation times 10 min and 9.5 h); lanes 6−9, RNase H activity toward the **α-L-LNA-1·T2** duplex (incubation times 10 min and 1, 4, and 9.5 h); lanes 10−13, RNase H activity toward the **LNA-1·T2** duplex (incubation times 10 min and 1, 4, and 9.5 h); lanes 14 and 15, RNase H activity toward the **LNA-2·T2** duplex (incubation times 10 min and 9.5 h).

Successful activation of RNase H by LNA mix-mers and gapmers has previously been reported,[31] for which reason the mix-mer **α-L-LNA-1** was compared with the analogous mix-mer **LNA-1**. As shown in Figure 10, cleavage of an 11-mer RNA strand when hybridized to either **DNA-1**, **α-L-LNA-1**, **LNA-1**, or **LNA-2** was studied. Lane 1 shows partial alkaline hydrolysis of the RNA strand. Lanes 2 and 3 show DNA/RNA (**DNA-1·T2**) control tubes without enzyme addition after 10 min and 9.5 h, respectively. Lanes 4 and 5 depict the rapid degradation of the unmodified DNA/RNA duplex after 10 min and 9.5 h, respectively. Lanes 6−9 show the progress in the cleavage of the **α-L-LNA-1·T2** duplex after 10 min and 1, 4, and 9.5 h, respectively, while no degradation could be detected for the analogous mix-mer **LNA-1** (lanes 10−13) within the same time intervals. The latter result contrasts with the previously reported RNA cleavage obtained with mix-mer LNAs of a different sequence.[31] A similar negative result was obtained for the fully modified **LNA-2** when hybridized to **T2** after 10 min and 9.5 h, as shown in lanes 14 and 15, respectively. The different mobility observed in lanes 14 and 15 can be attributed to migration of the RNA strand as complexes with LNA, despite the denaturing conditions of the gel. This emphasizes the indeed very strong hybridization between LNA and RNA. Identical cleavage patterns at different RNase H concentrations were obtained for **DNA-1** and the corresponding DNA with 5-methylcytosine nucleobases (**DNA-2**) (data not shown). Therefore any unfavorable interactions between oligonucleotides containing 5-methylcytosine nucleobases and the enzyme (data not shown) can be excluded, a result that supports the general applicability of 5-methylcytosine nucleobases for RNase H studies.

Discussion and Conclusion

With the procedures introduced earlier[4a,4c,4d,5] and herein, feasible synthetic routes toward the pyrimidine and purine α-L-LNA nucleoside derivatives have been developed. Oligomerization of the corresponding phosphoramidite derivatives on a DNA synthesizer at extended couplings times (∼10 min) allows the synthesis of mix-meric or fully modified α-L-LNA. The tautomeric characteristics of 5-methylcytosine nucleosides described above (Figure 2) are a point of concern when these are used as monomers in oligonucleotides because of the risk of conversion of the 5-methylcytosine moiety into a thymine moiety during the standard basic deprotection conditions used after automated oligonucleotide synthesis.[18] Because of the difference of only one mass unit between a 5-methylcytosine and a thymine base, MALDI-MS analysis does not reveal whether such a transformation has taken place for the oligomers **DNA-2**, **α-L-LNA-2**, and **LNA-2** studied herein. However, the obtained T_m values, especially as these follow the expected Watson−Crick base-pairing rules, strongly support the identity and stability of the 5-methylcytosine bases under the conditions used.[35]

The binding studies have demonstrated Watson−Crick hybridization of both mix-mer α-L-LNA and fully modified α-L-LNA toward both complementary DNA and RNA. High-affinity recognition comparable to that of LNA is possible toward complementary DNA only for fully modified α-L-LNA, whereas both mix-mer α-L-LNA and fully modified α-L-LNA display high binding affinity toward RNA (ΔT_m values per modification ca. +4 °C). We have shown earlier[4d] that α-L-LNA·α-L-LNA base pairing is very strong, as is LNA·LNA base pairing.[2d] Therefore, careful design of sequences or, alternatively, the use of mix-mer sequences is necessary for in vivo applications. It is thus a very important feature that it is possible to combine DNA monomers and α-L-LNA monomers in mix-mer α-L-LNA oligomers without compromising the high-affinity recognition of RNA. This fact, in combination with the relatively low affinity of mix-mer α-L-LNAs toward DNA, suggests (mix-mer) α-L-LNA to be potentially very attractive for antisense applications. Other important points that have been convincingly demonstrated herein are satisfactory discrimination between matched and mismatched complementary sequences and enhanced stability against 3′-exonucleolytic degradation.

An AON has basically two possible modes of action, which both involve hybridization to the RNA target. One is simply steric blocking of the mRNA; the other is recognition of the RNA·AON duplex as a substrate for the enzyme RNase H, which then cleaves the RNA strand of an RNA/DNA duplex. RNase H has been reported to bind in the minor groove of substrate RNA·DNA heteroduplexes adopting a duplex form intermediate between the A- and B-form, with a minor-groove width also intermediate between that of the A- and B-forms.[36] The furanose conformations in the RNA strand are of the *N*-type, whereas hybridization of a DNA strand to the RNA strand causes the furanose conformations of the DNA strand to change from the typical *S*-type (C2′-*endo*) into *E*-type conformations (O4′-*endo* range).[36] Thus, the activation of RNase H proposedly requires AONs with furanose rings able to adopt *E*-type (O4′-

(35) The potential conversion of 5-methylcytosine LNA monomers into thymine LNA monomers during the deprotection of LNA oligomers has been studied by analytical ion-exchange chromatography at pH 11, allowing a clear distinction between oligomers of identical sequence but with, e.g., one 5-methylcytosine monomer being converted into a thymine monomer (Daniel S. Pedersen, Anders M. Sørensen and Troels Koch personal communication; manuscript in preparation). The stability of 5-methylcytosine LNA monomers was confirmed (under deprotection conditions similar to those used herein), which further supports the assigned compositions of **DNA-2**, **α-L-LNA-2**, and **LNA-2**.

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endo), or perhaps *S*-type (C2′-*endo*), conformations. The locked furanose conformations of α-L-LNA might therefore explain the only limited ability of α-L-LNA•RNA duplexes to act as substrates for RNase H (high enzyme concentration and extended reaction time) despite the DNA-mimicking nature of α-L-LNA in α-L-LNA•RNA duplexes. In addition, the changed positioning of atoms and/or groups compared with natural DNA•RNA duplexes in the minor groove might contribute to the lack of efficient RNase H activation despite the additional 2′-*C*,4′-*C*-oxymethylene linker being conveniently accommodated on the brim of the major groove.[11]

Duplexes between RNA and arabinonucleic acids, an RNA stereoisomer, have been reported to be effecient substrates of RNase H.[37] It is noteworthy, however, that both stereoregular fully modified α-L-LNA and stereoirregular mix-mer α-L-LNA are able to induce RNase H cleavage at all when hybridized to RNA. Thus, whereas the backbone, e.g., the configurations at C3′ and C4′, is identical in RNA and arabinonucleic acids, it is, if the base is used as a point of reference, inverted at both C3′ and C4′ in α-L-LNA. The results obtained with α-L-LNA therefore not only substantiate the lack of stereoselectivity for Watson−Crick hybridization as demonstrated earlier, e.g., by the arabinonucleic acids[37] or the α-oligodeoxynucleotides,[38] but also question the (stereo)selectivity of nucleic acid−protein interactions.

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 organic phases were dried with Na_2SO_4, filtration was performed. After column chromatography, fractions containing product were pooled, evaporated under reduced pressure, and dried overnight under high vacuum to give the product unless otherwise specified. ^{1}H NMR spectra were recorded at 300 or 400 MHz, ^{13}C NMR spectra at 75.5 or 100.6 MHz, and ^{31}P NMR spectra at 121.5 MHz. Chemical shifts are reported in parts per million (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, when given, are based on 2D spectra and follow the standard carbohydrate/nucleoside nomenclature (the carbon atom of the 4′-*C*-substituent is numbered C-5″ even though the systematic compound names of the bicyclic structures are given according to the von Baeyer nomenclature). The assignments of methylene carbon and hydrogen atoms may, in general, be interchanged. Fast-atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode.

(1*S*,3*R*,4*S*,7*R*)-1-[[(4,4′-Dimethoxytrityl)oxy]methyl]-7-hydroxy-3-(5-methylcytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (7). To a stirred solution of nucleoside **5**[5] (300 mg, 0.52 mmol) in a mixture of anhydrous CH_2Cl_2 and anhydrous pyridine (20 mL, 3/2 v/v) was added chlorotrimethylsilane (0.13 mL, 1.04 mmol). After being stirred for 1 h at room temperature, the mixture was cooled to 0 °C and trifluoromethanesulfonic anhydride (0.26 mL, 1.56 mmol) was added dropwise over the course of 10 min. The mixture was allowed to warm to room temperature, and after 18 h analytical TLC (CH_2Cl_2/MeOH/pyridine, 89.8/10.0/0.2 v/v/v) indicated complete conversion to a single product with very low mobility. The mixture was poured into a solution of saturated NH_3 in MeOH (30 mL) and the mixture was kept in a sealed flask at room temperature overnight. Evaporation to dryness under reduced pressure followed by silica gel column chromatography with CH_2Cl_2/MeOH/pyridine (0−5% MeOH and 0.5% pyridine by volume) as eluent yielded nucleoside **7** (241 mg, 81%) as an off-white solid material. R_f 0.16 (CH_2Cl_2/MeOH/pyridine, 89.8/10.0/0.2 v/v/v); FAB-MS *m*/*z* 572 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.62−7.22 (m, 10H), 6.86 (d, 4H, J = 7.9 Hz), 5.98 (s, 1H), 4.77 (s, 1H), 4.48 (s, 1H), 4.25−4.00 (m, 2H), 3.80−3.45 (m, 8H), 1.90 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 165.1, 158.3, 156.4, 144.5, 137.6, 135.4, 135.2, 129.8, 127.8, 127.6, 126.6, 113.0, 101.3, 89.5, 87.9, 85.9, 79.0, 73.5, 73.0, 60.4, 54.9, 13.2.

(1*S*,3*R*,4*S*,7*R*)-3-(4-*N*-Benzoyl-5-methylcytosin-1-yl)-1-[[(4,4′-dimethoxytrityl)oxy]methyl]-7-hydroxy-2,5-dioxabicyclo[2.2.1]-heptane (8). To a solution of nucleoside **7** (90 mg, 0.16 mmol) in anhydrous pyridine (5 mL) was added chlorotrimethylsilane (0.04 mL, 0.31 mmol), and the mixture was stirred for 1.5 h at room temperature. *N*-Benzoyl-1*H*-tetrazole[17] (42 mg, 0.24 mmol) was added and stirring was continued for 12 h at room temperature. H_2O (10 mL) was added, and after being stirred for an additional 5 h at room temperature, the mixture was diluted with CH_2Cl_2 (100 mL), washed with a saturated aqueous solution of $NaHCO_3$ (3 × 100 mL), 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, 0−4% MeOH and 0.5% pyridine by volume) to give nucleoside **8** as a white solid material (86 mg, 80%). R_f 0.61 (CH_2Cl_2/MeOH/pyridine, 89.8/10.0/0.2 v/v/v); FAB-MS *m*/*z* 676 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.32 (d, 2H, J = 7.3 Hz, Bz), 7.74 (s, 1H, 6-H), 7.55−7.22 (m, 12H, DMT, Bz), 6.86 (d, 4H, J = 8.5 Hz, DMT), 5.99 (s, 1H, 1′-H), 4.59 (s, 1H, 2′-H), 4.42 (s, 1H, 3′-H), 4.13 (d, 1H, J = 9.1 Hz, 5′-H_a), 4.07 (d, 1H, J = 9.1 Hz, 5′-H_b), 3.79 (s, 6H, OCH_3), 3.59 (d, 1H, J = 10.8 Hz, 5″-H_a), 3.52 (d, 1H, J = 10.8 Hz, 5”-H_b), 2.19 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 179.0 (C═O), 159.7 (C-4), 158.4 (DMT), 147.8 (C-2), 144.2, 135.2, 132.3, 129.9, 129.7, 128.0, 127.8, 126.8 (DMT, Bz), 136.9 (C-6), 113.1 (DMT), 110.4 (C-5), 89.7, 86.2 (C-4′, DMT), 87.7 (C-1′), 78.7 (C-2′), 74.1 (C-3′), 72.7 (C-5′), 59.8 (C-5″), 55.1 (DMT), 13.8 (CH_3). The ^{1}H NMR data obtained are in agreement with the ^{1}H NMR data obtained for the corresponding enantiomeric derivative (unpublished results, Poul Nielsen, Department of Chemistry, University of Southern Denmark).

(1*S*,3*R*,4*S*,7*R*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-[[(4,4′-dimethoxytrityl)oxy]methyl]-3-(4-*N*-benzoyl-5-methylcytosin-1-yl)-2,5-dioxabicyclo[2.2.1]heptene (9). To a stirred solution of nucleoside **8** (255 mg, 0.38 mmol) in anhydrous CH_2Cl_2 (8 mL) at 0 °C was added (*N*,*N*-diisopropyl)ethylamine (0.20 mL, 1.13 mmol). After dropwise addition of 2-cyanoethyl [(*N*,*N*-diisopropyl)phosphoramido]-chloridite (0.13 mL, 0.57 mmol), the mixture was allowed to warm to room temperature and stirring was continued for 12 h. MeOH (0.1 mL) was added, and the mixture was diluted with CH_2Cl_2 (30 mL), washed with a saturated aqueous solution of $NaHCO_3$ (3 × 30 mL), and dried (Na_2SO_4). The organic phase was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography with EtOAc/*n*-hexane/NEt_3 (49.5/49.5/1.0 v/v/v) as eluent to give amidite **9** as a white foam (148 mg, 45%). R_f 0.88 (CH_2Cl_2/MeOH/pyridine, 89.8/10.0/0.2 v/v/v); ^{31}P NMR [$(CD_3)_2SO$] δ 150.5, 150.4.

9-[2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-[(benzoyloxy)methyl]-3-*O*-benzyl-α-L-*threo*-pentofuranosyl]-6-*N*-benzoyladenine (11). Furanose **10**[12] (2.05 g, 3.65 mmol) was dissolved in anhydrous acetonitrile (30 mL). 6-*N*-Benzoyladenine (1.86 g, 7.78 mmol) and $SnCl_4$ (1.30 mL, 11.11 mmol) were added, and the mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of a saturated aqueous solution of $NaHCO_3$ until the evolution of CO_2 had ceased, whereupon

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the mixture was filtered through a layer of Celite 545. The layer of Celite 545 was washed with CH_2Cl_2 (2 × 50 mL) and the combined filtrates were washed successively with a saturated aqueous solution of $NaHCO_3$ (3 × 150 mL) and H_2O (2 × 150 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with EtOAc/petroleum ether (40−60% EtOAc by volume) as eluent to give nucleoside **11** as a white solid material (1.40 g, 52%). R_f 0.40 (MeOH/CH_2Cl_2, 2/23 v/v); FAB-MS *m*/*z* 742 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.60 (br s, 1H, 6-H), 8.73 (s, 1H, 8-H), 8.51 (s, 1H, 2-H), 8.02−7.98 (m, 4H), 7.89−7.86 (m, 2H), 7.58−7.16 (m, 14H), 6.58 (d, 1H, J = 2.6 Hz, 1′-H), 5.88 (t, 1H, J = 2.2 Hz, 2′-H), 4.93 (d, 1H, J = 12.0 Hz, Bn), 4.79 (d, 1H, J = 12.0 Hz, Bn), 4.70−4.61 (m, 4H), 4.34 (d, 1H, J = 1.8 Hz, 3′-H), 2.17 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$) δ 169.3, 165.5, 165.3, 152.3, 151.3, 149.4, 135.5, 133.3, 133.1, 132.9, 132.3, 129.3, 129.3, 129.1, 128.7, 128.3, 128.2, 128.1, 128.1, 128.0, 128.0, 127.6, 122.8 (Bn, Bz, Ac, C-4, C-5, C-6, C-8), 141.1 (C-2), 87.2, 87.0 (C-1′, C-4′), 80.8 (C-3′), 80.1 (C-2′), 72.2 (Bn), 62.7, 62.3 (C-5′, C-5″), 20.5 (Ac).

9-[2-*O*-Acetyl-5-*O*-benzoyl-4-*C*-[(benzoyloxy)methyl]-3-*O*-benzyl-α-L-*erythro*-pentofuranosyl]-6-*N*-benzoyladenine (14). To a solution of nucleoside **11** (3.63 g, 4.90 mmol) in MeOH (75 mL) was added a saturated solution of NH_3 in MeOH (75 mL). The solution was stirred at 0 °C for 2 h and evaporated to dryness under reduced pressure. The residue was coevaporated with anhydrous toluene (2 × 40 mL) and dissolved in a mixture of anhydrous CH_2Cl_2 (100 mL) and anhydrous pyridine (20 mL). After the mixture was cooled to −30 °C, trifluoromethanesulfonic anhydride (1.7 mL, 10.3 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature, and after 1.5 h additional trifluoromethanesulfonic anhydride (0.5 mL, 3.03 mmol) was added. After being stirred for an additional 30 min, the mixture was diluted with CH_2Cl_2 (130 mL), washed with a saturated aqueous solution of $NaHCO_3$ (3 × 200 mL) and dried (Na_2SO_4). After evaporation of the solvents, the residue was coevaporated with anhydrous toluene (2 × 75 mL) and dissolved in anhydrous toluene (150 mL). KOAc (2.45 g, 25.0 mmol) and 18-crown-6 (2.56 g, 9.70 mmol) were added, and the mixture was stirred for 30 min at room temperature and then heated to 80 °C for 30 min. The mixture was cooled to room temperature and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with MeOH/CH_2Cl_2 (0−2% MeOH by volume) as eluent, affording nucleoside **14** (3.05 g, 84%) as a white solid material. R_f 0.44 (MeOH/CH_2Cl_2, 1/19 v/v); FAB-MS *m*/*z* 742 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.00 (br s, 1H, 6-NH), 8.76 (s, 1H, 8-H), 8.59 (s, 1H, 2-H), 8.05−7.98 (m, 6H), 7.64−7.41 (m, 10H), 7.28−7.23 (m, 4H), 6.77 (d, 1H, J = 4.6 Hz, 1′-H), 5.86 (m, 1H, H-2′), 5.23 (d, 1H, J = 12.3 Hz), 4.74−4.66 (m, 3H), 4.56−4.50 (m, 3H), 2.04 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$) δ 168.8, 165.8, 142.7, 136.0, 133.5, 133.3, 132.7, 129.6, 129.6, 128.8, 128.6, 128.5, 128.4, 128.4, 128.1, 127.8 (Bn, Bz, Ac, C-2, C-4, C-5, C-6, C-8), 83.8 (C-4′), 82.2 (C-1′), 78.4 (C-3′), 74.3 (Bn), 70.8 (C-2′), 64.7, 63.4 (C-5′, C-5″), 20.5 (Ac).

9-[3-*O*-Benzyl-4-*C*-(hydroxymethyl)-α-L-*erythro*-pentofuranosyl]-6-*N*-benzoyladenine (15). Nucleoside **14** (1.09 g, 1.47 mmol) was dissolved in a saturated solution of NH_3 in MeOH (40 mL). After the mixture was stirred for 30 min at room temperature, an aqueous solution of NH_3 (10 mL, 32% by weight) was added. After 22 h, the mixture was evaporated to dryness under reduced pressure and the residue, after coevaporation with anhydrous pyridine (3 × 20 mL), was dissolved in anhydrous pyridine (20 mL). Chlorotrimethylsilane (2.8 mL, 22 mmol) was added and stirring was continued for 2.5 h at room temperature. The reaction mixture was cooled to 0 °C, BzCl (0.9 mL, 7.8 mmol) was added, and stirring was continued for 16.5 h at room temperature. After the mixture was cooled to 0 °C, water (40 mL) was added and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in H_2O/MeOH/saturated methanolic ammonia (65 mL, 3/4/6 v/v/v), and the mixture was stirred for 6.5 h at room temperature and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with MeOH/CH_2Cl_2 (4−6% MeOH by volume) as eluent, yielding nucleoside **15** (656 mg, 91%) as a white foam, which was used in the next step without further purification. R_f 0.47 (MeOH/CH_2Cl_2, 1/9); FAB-MS *m*/*z* 492 $[M + H]^+$; ^{1}H NMR [$(CD_3)_2SO$] δ 11.18 (br s, 1H, 6-NH), 8.75 (s, 1H, 2-H), 8.66 (s, 1H, 8-H), 8.07−7.95 (m, 2H), 7.67−7.29 (m, 8H), 6.48 (d, 1H, J = 4.8 Hz, 1′-H), 5.92 (br s, 1H, 2′-OH), 5.35 (br s, 1H), 5.09 (br s, 1H), 4.81 (d, 1H, J = 11.6 Hz), 4.67 (m, 1H, 2′-H), 4.62 (d, 1H, J = 11.6 Hz) 4.40 (d, 1H, J = 5.0 Hz, 3′-H), 3.70 (d, 1H, J = 11.6 Hz), 3.63 (d, 1H, J = 11.5 Hz), 3.54 (s, 2H); ^{13}C NMR [$(CD_3)_2$-SO] δ 164.7, 151.5, 150.1, 138.4, 133.5, 132.8, 132.7, 129.3, 128.6, 128.5, 128.3, 127.6, 127.5, 125.1 (Bn, Bz, C-4, C-5, C-6), 152.3 (C-2), 144.4 (C-8), 110.4, 87.8 (C-4′), 83.7 (C-1′), 79.4 (C-3′), 72.5 (Bn), 69.9 (C-2′), 63.3, 61.1 (C-5′, C-5″).

9-[3-*O*-Benzyl-5-*O*-(methanesulfonyl)-4-*C*-[[(methanesulfonyl)-oxy]methyl]-α-L-*erythro*-pentofuranosyl]-6-*N*-benzoyladenine (16). Nucleoside **15** (1.75 g, 3.57 mmol) was coevaporated with anhydrous pyridine (100 mL) and dissolved in anhydrous pyridine (100 mL). The mixture was cooled to 0 °C and methanesulfonyl chloride (0.61 mL, 7.9 mmol) was added dropwise. After the mixture was stirred for 2 h, H_2O (10 mL) was added and the mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (75 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (3 × 75 mL) and dried (Na_2SO_4). The organic phase was evaporated to dryness under reduced pressure and the residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (2−4% MeOH by volume) as eluent, affording nucleoside **16** (1.83 g, 79%) as a white solid material. R_f 0.63 (MeOH/CH_2Cl_2, 1/9 v/v); FAB-MS *m*/*z* 648 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.10 (s, 1H, 6-NH), 8.52 (s, 1H, 8-H), 8.48 (s, 1H, 2-H), 7.96−7.95 (m, 2H), 7,62−7.31 (m, 8H), 6.46 (d, 1H, J = 3.7 Hz, 1′-H), 5.42 (br s, 1H, 2′-OH), 5.04 (d, 1H, J = 11.7 Hz, 3′-H), 4.79 (d, 1H, J = 11.7 Hz, Bn), 4.68 (br s, 1H, 2′-H), 4.58 (d, 1H, J = 11.7 Hz, Bn), 4.45−4.26 (m, 4H, 5′-H, 5″-H), 3.03 (s, 3H, Ms), 3.01 (s, 3H, Ms); ^{13}C NMR ($CDCl_3$) δ 174.3, 164.8, 151.6, 148.5, 136.3, 133.4, 132.9, 128.9, 128.8, 128.7, 128.5, 127.9, 121.8 (Bn, Bz, C-4, C-5, C-6), 152.1 (C-2), 143.5 (C-8), 84.8 (C-1′), 82.4 (C-4′), 78.8, 68.3 (C-5′, C-5″), 73.6 (Bn), 70.3 (C-2′), 69.0 (C-3′), 37.7 (Ms), 37.6 (Ms).

(1*S*,3*R*,4*S*,7*R*)-1-(Acetoxymethyl)-3-(6-*N*-benzoyladenin-9-yl)-7-benzyloxy-2,5-dioxabicyclo[2.2.1]heptane (18). Nucleoside **16** (1.31 g, 2.02 mmol) was coevaporated with anhydrous toluene (50 mL) and dissolved in anhydrous THF (50 mL). The mixture was cooled to 0 °C and NaH [60% suspension in mineral oil (by weight), 175 mg, 4.38 mmol] was added. After being stirred at 0 °C for 6.5 h, the reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in CH_2Cl_2 (100 mL). Washing was performed with a saturated aqueous solution of $NaHCO_3$ (3 × 100 mL) and the organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was dissolved in 1,4-dioxane (50 mL), and KOAc (1.02 g, 10.4 mmol) and 18-crown-6 (1.08 g, 4.09 mmol) were added. The mixture was heated under reflux for 19 h and subsequently evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (0.5% MeOH by volume) as eluent, yielding nucleoside **18** (911 mg, 87%) as a white solid material, which was used in the next step without further purification. R_f 0.60 (MeOH/CH_2Cl_2, 1/9 v/v); FAB-MS *m*/*z* 516 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.37 (br s, 1H, 6-NH), 8.72 (s, 1H, 8-H), 8.51 (s, 1H, 2-H), 8.04−8.02 (m, 2H), 7.60−7.33 (m, 8H), 6.46 (s, 1H, 1′-H), 4.76 (d, 1H, J = 11.9 Hz, Bn), 4.66 (d, 1H, J = 11.7 Hz, Bn), 4.65 (s, 1H, 2′-H), 4.50 (d, 1H, J = 12.6 Hz, 5″-H), 4.38 (d, 1H, J = 12.6 Hz, 5″-H), 4.33 (s, 1H, 3′-H), 4.30 (d, 1H, J = 8.4 Hz, 5′-H), 4.11 (d, 1H, J = 8.2 Hz, 5′-H), 2.06 (s, 3H, Ac); ^{13}C NMR ($CDCl_3$) δ 170.1 (Ac), 164.3, 152.4, 151.9, 149.5, 141.2, 136.4, 133.5, 132.5, 128.6, 128.5, 128.2, 127.8, 127.6, 122.8, (C-2, C-4, C-5, C-6, C-8, Bz, Bn), 87.0 (C-4′), 84.5 (C-1′), 79.7 (C-3′), 77.1 (C-2′), 73.5 (C-5′), 72.3 (Bn), 59.7 (C-5″), 20.5 (Ac).

(1*R*,3*R*,4*S*,7*R*)-3-(6-*N*-Benzoyladenin-9-yl)-7-benzyloxy-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (19). To a solution of nucleoside **18** (1.13 g, 2.19 mmol) in MeOH (25 mL) was added a saturated solution of NH_3 in MeOH (25 mL). The reaction mixture was stirred at 0 °C for 17 h and evaporated to dryness. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (1−5% MeOH by volume) as eluent, affording nucleoside **19** (862 mg, 83%) as a white solid material. R_f 0.22 (MeOH/CH_2Cl_2, 2/23 v/v); FAB-MS *m*/*z* 474 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.20 (br s, 1H, 6-NH), 8.74 (s, 1H, 8-H), 8.51 (s, 1H, 2-H), 8.04−8.01 (m, 2H), 7.60−7.27 (m, 8H), 6.47 (s, 1H, 1′-H), 4.79 (d, 1H, J = 11.9 Hz), 4.69 (d, 1H, J = 11.9 Hz), 4.62 (s, 1H, 2′-H), 4.37 (s, 1H, 3′-H), 4.23 (d, 1H, J = 8.1 Hz), 4.04 (d, 1H, J = 8.4 Hz), 3.99−3.89 (m, 3H, 5″-H, 5″-OH); ^{13}C NMR ($CDCl_3$) δ 164.8, 151.6, 149.3, 141.3, 141.3, 136.7, 133.4, 132.5, 128.5, 128.4, 128.0, 127.8, 127.5, 122.2 (C-4, C-5, C-6, C-8, Bz, Bn), 152.4 (C-2), 89.9 (C-4′), 84.5 (C-1′), 79.3 (C-3′), 77.0 (C-2′), 73.3 (C-5′), 72.3 (Bn), 57.8 (C-5″).

(1*R*,3*R*,4*S*,7*R*)-3-(6-*N*-Benzoyladenin-9-yl)-7-hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (20) and (1*R*,3*R*,4*S*,7*R*)-3-(Adenin-9-yl)-7-hydroxy-1-(hydroxymethyl)-2,5-dioxabicyclo[2.2.1]heptane (21). To a solution of nucleoside **19** (503 mg, 1.06 mmol) in MeOH (40 mL) was added a catalytical amount of Pd/C (10% by weight) and $HCOONH_4$ (201 mg, 3.52 mmol). The mixture was heated under reflux for 12 h and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with CH_2Cl_2/MeOH (3−8% MeOH by volume) as eluent, yielding nucleosides **20** (177 mg, 44%) and **21** (72 mg, 24%), both as white solid materials. Data for **20**: R_f 0.23 (MeOH/CH_2Cl_2, 13/87 v/v); FAB-MS *m*/*z* 384 $[M + H]^+$; ^{1}H NMR [(CD_3)SO] δ 11.10 (br s, 1H, 6-NH), 8.72 (s, 1H, 2-H), 8.65 (s, 1H, 8-H), 8.04 (d, 2H, J = 8.2 Hz, Bz), 7.65−7.51 (m, 3H, Bz), 6.51 (s, 1H, 1′-H), 5.94 (d, 1H, J = 4.4 Hz, 3′-OH), 4.93 (t, 1H, J = 5.6 Hz, 5″-OH), 4.44−4.42 (m, 2H, 2′-H, 3′-H), 4.09 (d, 1H, J = 8.2 Hz, 5′-H), 4.02 (d, 1H, J = 8.2 Hz, 5′-H), 3.73 (d, 2H, J = 5.5 Hz, 5″-H); ^{13}C NMR [(CD_3)SO] δ 152.2, 151.6, 150.3, 133.4, 132.5, 128.5, 125.2 (Bz, C-2, C-4, C-5, C-6), 142.5 (C-8), 90.7 (C-4′), 84.4 (C-1′), 79.4 (C-2′), 72.9 (C-3′), 72.6 (C-5′), 57.5 (C-5″). Data for **21**: R_f 0.12 (MeOH/CH_2Cl_2, 13/87 v/v); FAB-MS *m*/*z* 280 $[M + H]^+$; ^{1}H NMR [(CD_3)SO] δ 8.34 (s, 1H, 2-H), 8.11 (s, 1H, 8-H), 6.32 (s, 1H, 1′-H), 5.90 (br s, 1H, 3′-OH), 4.90 (br s, 1H, 5″-OH), 4.36 (s, 1H, 3′-H), 4.32 (s, 1H, 2′-H), 4.03 (d, 1H, J = 8.1 Hz, 5′-H), 3.99 (d, 1H, J = 8.3 Hz, 5′-H), 3.69 (s, 2H, 5″-H); ^{13}C NMR [(CD_3)SO] δ 156.1 (C-6), 152.8 (C-2), 149.6 (C-4), 139.1 (C-8), 128.7, 118.5 (C-5), 90.6 (C-4′), 84.0 (C-1′), 79.6 (C-2′), 73.1 (C-3′), 72.7 (C-5′), 57.7 (C-5″).

(1*S*,3*R*,4*S*,7*R*)-1-[[(4,4′-Dimethoxytrityl)oxy]methyl]-7-hydroxy-3-(6-*N*-benzoyladenin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (22). Compound **20** was coevaporated with anhydrous pyridine (30 mL, 12 mL) and dissolved in anhydrous pyridine (6 mL) at room temperature, whereupon 4,4′-dimethoxytrityl chloride (170 mg, 0.53 mmol) was added. After being stirred for 11 h, the mixture was poured into petroleum ether/CH_2Cl_2/H_2O (30 mL, 1/1/1 v/v/v), and the organic phase was separated, washed with a saturated aqueous solution of $NaHCO_3$ (3 × 15 mL), and evaporated to dryness under reduced pressure. The residue was coevaporated with anhydrous toluene (6 mL) and purified by silica gel column chromatography with CH_2Cl_2/MeOH/pyridine (0−2.5% MeOH and 0.5% pyridine by volume) as eluent, affording nucleoside **22** (147 mg, 82%) as a white solid material. R_f 0.46 (MeOH/CH_2Cl_2, 1/9 v/v); FAB-MS *m*/*z* 686 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 9.35 (br s, 1H, 6-NH), 8.72 (s, 1H, 2-H), 8.52 (s, 1H, 8-H), 8.01−7.99 (m, 2H), 7.56−7.14 (m, 12H), 6.82−6.79 (m, 4H), 6.52 (s, 1H, 1′-H), 4.58−4.56 (m, 2H), 4.21−4.19 (m, 2H), 4.14 (d, 1H, J = 8.6 Hz, 5′-H), 3.75 (s, 6H, OCH_3), 3.52 (s, 2H, 5″-H); ^{13}C NMR ($CDCl_3$) δ 164.7, 158.4, 149.2, 144.2, 135.2, 135.1, 133.4, 132.6, 129.8, 128.9, 128.6, 128.0, 127.8, 127.8, 127.8, 126.8, 125.1, 122.4, 113.1 (DMT, C-4, C-5, C-6, Bz), 152.4 (C-2), 141.4 (C-8), 89.2, 86.3 (DMT, C-4′), 84.6 (C-1′), 79.3, 74.6 (C-2′, C-3′), 73.2 (C-5′), 60.0 (C-5″), 55.1 (DMT).

(1*S*,3*R*,4*S*,7*R*)-7-[2-Cyanoethoxy(diisopropylamino)phosphinoxy]-1-[[(4,4′-dimethoxytrityl)oxy]methyl]-3-(6-*N*-benzoyladenin-9-yl)-2,5-dioxabicyclo[2.2.1]heptane (23). Nucleoside **22** (145 mg, 0.21 mmol) was coevaporated with anhydrous pyridine (2 × 3 mL) and dissolved in anhydrous CH_2Cl_2 (5 mL). *N*,*N*-Diisopropylethylamine (0.15 mL) was added and the solution was cooled to 0 °C, whereupon 2-cyanoethyl (*N*,*N*-diisopropyl)phosphoramidochloridite (0.10 mL, 0.52 mmol) was added. The mixture was stirred in the dark for 14.5 h at room temperature, CH_2Cl_2 (45 mL) was added, and the mixture was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 35 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with *n*-hexane/EtOAc/triethylamine (5/4/1 v/v/v) as eluent, affording amidite **23** (118 mg, 63%) as a white foam. R_f 0.70, 0.77 (MeOH/CH_2Cl_2, 2/23 v/v); ^{31}P NMR δ (CH_3CN) 150.2, 150.0.

3-*O*-Benzyl-5-*O*-(methanesulfonyl)-4-*C*-[[(methanesulfonyl)oxy]-methyl]-1,2-*O*-isopropylidene-*β*-L-*threo*-pentofuranose (25). Compound **24**[24] (2.00 g, 6.40 mmol) was coevaporated with anhydrous pyridine (20 mL) and dissolved in anhydrous pyridine (15 mL). The solution was stirred and cooled to 0 °C, whereupon methanesulfonyl chloride (1.25 mL, 16.1 mmol) was added dropwise. The mixture was allowed to warm to room temperature, and after it was stirred for 2 h, additional methanesulfonyl chloride (1.00 mL, 12.9 mmol) was added. After another 2 h, ice-cold H_2O (40 mL) was added, and extraction was performed with diethyl ether (2 × 150 mL). The combined organic phase was washed with brine (100 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with MeOH/CH_2Cl_2 (1/99 v/v) as eluent, giving furanose **25** (2.00 g, 66%) as a white semisolid material, which was used in the next step without further purification. FAB-MS *m*/*z* 467 $[M + H]^+$; ^{1}H NMR ***δ*** ($CDCl_3$) 7.37 (s, 5H), 5.80 (d, 1H, J = 3.3 Hz), 4.89 (d, 1H, J = 11.7 Hz), 4.79 (d, 1H, J = 11.7 Hz), 4.67 (t, 1H, J = 4.4 Hz), 4.59 (d, 1H, J = 11.8 Hz), 4.44 (d, 1H, J = 12.1 Hz), 4.34 (d, 1H, J = 11.0 Hz), 4.21−4.13 (m, 2H), 3.10 (s, 3H), 2.98 (s, 3H), 2.16 (s, 3H), 1.68 (s, 3H), 1.34 (s, 3H); ^{13}C NMR δ ($CDCl_3$) 136.5, 128.4, 128.2, 128.2, 128.1, 127.9, 113.8, 104.3, 83.0, 78.2, 77.6, 72.6, 69.3, 68.5, 37.8, 37.2, 26.0, 25.4.

1,2-Di-*O*-acetyl-3-*O*-benzyl-5-*O*-(methanesulfonyl)-4-*C*-[[(methanesulfonyl)oxy]methyl]-L-*threo*-pentofuranose (26). Compound **25** (5.00 g, 10.7 mmol) was dissolved in 80% TFA (40 mL). After being stirred for 4 h at room temperature, the mixture was evaporated to dryness under reduced pressure. The residue was coevaporated with anhydrous pyridine (50 mL) and dissolved in anhydrous pyridine (50 mL), whereupon Ac_2O (4.0 mL, 42.0 mmol) was added. The mixture was stirred for 3 h at room temperature, a saturated aqueous solution of $NaHCO_3$ (150 mL) was added, and extraction was performed with CH_2Cl_2 (3 × 200 mL). The combined organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with CH_2Cl_2 as eluent, affording furanose **26** (3.50 g, 63%, 4/5 anomeric mixture) as a white solid material. FAB-MS *m*/*z* 511 $[M + H]^+$; ^{13}C NMR δ ($CDCl_3$) 169.5, 169.5, 169.1, 136.6, 136.5, 128.7, 128.6, 128.4, 128.3, 128.1, 99.5, 92.3, 86.5, 82.8, 81.5, 81.2, 79.8, 76.3, 73.7, 72.8, 68.8, 68.6, 67.9, 66.7, 37.9, 37.8, 37.7, 37.5, 21.0, 20.9, 20.7, 20.4.

9-[2-*O*-Acetyl-3-*O*-benzyl-5-*O*-(methanesulfonyl)-4-*C*-[[(methanesulfonyl)oxy]methyl]-α-L-*threo*-pentofuranosyl]-6-*N*-benzoyladenine (27). Furanose **26** (6.20 g, 12.1 mmol) was coevaporated with anhydrous CH_3CN (50 mL) and dissolved in anhydrous CH_3CN (125 mL) under stirring at room temperature. 6-*N*-Benzoyladenine (7.20 g, 30.1 mmol) and then $SnCl_4$ (5.0 mL, 42.7 mmol) were added. Shortly thereafter the mixture turned clear, and after it was stirred for 1.5 h, additional $SnCl_4$ (3.2 mL, 27.3 mmol) was added. After 4.5 h, a saturated aqueous solution of $NaHCO_3$ was added slowly until CO_2 evolution ceased. The mixture was filtered through Celite 545 three times [after each filtration the Celite 545 was washed MeOH/CH_2Cl_2 (500 mL, 8/2 v/v)]. The combined filtrate was evaporated to dryness

under reduced pressure and redissolved in CH_2Cl_2 (300 mL), washed successively with saturated aqueous solutions of $NaHCO_3$ (2 × 100 mL) and brine (2 × 100 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with MeOH/CH_2Cl_2 (1.5/98.5 v/v) as eluent, yielding nucleoside **27** (4.80 g, 57%) as a white solid material. FAB-MS *m*/*z* 690 $[M + H]^+$; ^{1}H NMR δ ($CDCl_3$) 9.11 (s, 1H), 8.78 (s, 1H), 8.38 (s, 1H), 8.03 (d, 2H, J = 7.5 Hz), 7.61−7.27 (m, 8H), 6.45 (d, 1H, J = 2.7 Hz), 5.94 (t, 1H, J = 2.4 Hz), 4.81−4.65 (m, 3H), 4.43−4.28 (m, 4H), 3.00 (s, 3H), 2.96 (s, 3H), 2.16 (s, 3H); ^{13}C NMR δ ($CDCl_3$) 169.5, 164.6, 152.9, 151.6, 149.7, 141.2, 135.7, 133.4, 132.8, 128.8, 128.8, 128.7, 128.6, 128.5, 128.4, 128.0, 127.8, 123.0, 87.3, 86.0, 81.3, 79.4, 73.3, 67.4, 65.5, 37.7, 37.6, 20.7.

9-(3-*O*-Benzyl-5-*O*-(methanesulfonyl)-4-*C*-[[(methanesulfonyl)-oxy]methyl]-α-L-*threo*-pentofuranosyl)-6-*N*-benzoyladenine (28). Nucleoside **27** (2.10 g, 3.00 mmol) was dissolved in a mixture of MeOH (35 mL) and saturated methanolic ammnonia (15 mL). The mixture was stirred at room temperature for 1.5 h and then evaporated to dryness under reduced pressure. The residue was coevaporated with 96% EtOH (50 mL) and purified by silica gel column chromatography with MeOH/CH_2Cl_2 (1/40 v/v) as eluent, affording nucleoside **28** (1.56 g, 79%) as a white solid material. FAB-MS *m*/*z* 648 $[M + H]^+$; ^{1}H NMR δ ($CDCl_3$) 9.07 (s, 1H), 8.46 (s, 1H), 8.30 (s, 1H), 7.96 (d, 2H, J = 6.9 Hz), 7.60−7.25 (m, 9H), 6.12 (d, 1H, J = 2.4 Hz), 5.30 (d, 1H, J = 2.4 Hz), 5.01 (dd, 1H, J = 11.4, 2.4 Hz), 4.79 (dd, 1H, J = 11.4, 2.7 Hz), 4.63 (dd, 1H, J = 10.5, 2.7 Hz) 4.46−4.24 (m, 4H), 3.03 (s, 3H), 2.89 (s, 3H); ^{13}C NMR $\boldsymbol{\delta}$ ($CDCl_3$) 165.0, 152.4, 151.2, 148.5, 142.0, 136.9, 133.3, 133.0, 129.0, 128.6, 128.5, 128.3, 127.8, 121.7, 87.4, 82.8, 81.8, 78.6, 73.2, 68.5, 68.3, 37.7, 37.4.

9-(2-*O*-Acetyl-3-*O*-benzyl-5-*O*-(methanesulfonyl)-4-*C*-[[(methanesulfonyl)oxy]methyl]-α-L-*erythro*-pentofuranosyl)-6-*N*-benzoyladenine (29). Nucleoside **28** (1.40 g, 2.16 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (50 mL) and anhydrous pyridine (10 mL). The stirred solution was cooled to −30 °C and trifluoromethanesulfonic anhydride (0.80 mL, 4.80 mmol) was added. After 1.5 h, the reaction mixture was allowed to warm to 0 °C, additional trifluoromethanesulfonic anhydride (0.20 mL, 1.20 mmol) was added, and stirring at 0 °C was continued for 2.5 h. A saturated aqueous solution of $NaHCO_3$ (10 mL) was added, followed by addition of CH_2Cl_2 (150 mL). The organic phase was separated, washed with a saturated aqueous solution of $NaHCO_3$ (3 × 70 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of anhydrous toluene (100 mL) and anhydrous CH_2Cl_2 (20 mL), and KOAc (1.20 g, 12.2 mmol) and 18-crown-6 (1.20 g, 4.50 mmol) were added at room temperature under stirring. The temperature was raised to 50 °C and stirring was continued for 16 h. After the mixture was cooled to room temperature, CH_2Cl_2 (200 mL) was added, and the resulting mixture was washed with a saturated aqueous solution of $NaHCO_3$ (3 × 90 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography with MeOH/CH_2Cl_2 (1−2% MeOH by volume) as eluent, affording nucleoside **29** (1.05 g, 70%) as a white solid material. FAB-MS *m*/*z* 690 $[M + H]^+$; ^{1}H NMR δ ($CDCl_3$) 9.17 (s, 1H), 8.74 (s, 1H), 8.57 (s, 1H), 8.04 (d, 2H, J = 7.2 Hz), 7.61−7.26 (m, 9H), 6.74 (d, 1H, J = 3.9 Hz), 5.74 (s, 1H), 4.90−4.64 (m, 4H), 4.58 (d, 1H, J = 10.8 Hz), 4.40 (d, 1H, J = 10.8 Hz), 4.28 (d, 1H, J = 10.8 Hz), 3.04 (s, 3H), 3.02 (s, 3H), 2.03 (s, 3H); ^{13}C NMR δ ($CDCl_3$) 168.6, 164.6, 152.8, 151.7, 149.5, 142.3, 135.9, 132.8, 128.8, 128.8, 128.7, 128.5, 128.4, 127.8, 122.4, 82.4, 82.3, 78.2, 74.6, 70.4, 68.0, 67.9, 38.0, 37.7, 20.5.

Alternative Synthesis of 16. Nucleoside **29** (1.00 g, 1.45 mmol) was dissolved in a mixture of MeOH (35 mL) and saturated methanolic ammnonia (15 mL). The mixture was stirred for 3.5 h at room temperature and then evaporated to dryness under reduced pressure. The residue was coevaporeted with EtOH (50 mL) and purified by silica gel column chromatography with CH_2Cl_2/MeOH (2−4% MeOH by volume) as eluent, yielding nucleoside **16** (0.80 g, 85%) as a white solid material. Analytical data were identical with those reported above for **16**.

Synthesis, Deprotection, and Purification of Oligonucleotides. All LNA and α-L-LNA oligomers were prepared by the phosphoramidite approach as described earlier[2a,4] on a Biosearch 8750 DNA synthesizer, generally on CPG solid supports. The stepwise coupling efficiencies for α-L-LNA phosphoramidite **6** (with 10 min coupling time) and unmodified deoxynucleoside phosphoramidites (with 2 min standard coupling time) were generally >99% with 1*H*-tetrazole as activator. The stepwise coupling yield for synthesis of the 11-mix-mer **α-L-LNA-1** was 81−96% for amidite **23** (with 10 min coupling time). For synthesis of the fully modified **α-L-LNA-2**, a universal CPG support (BioGenex) was applied. The stepwise coupling yields obtained were 85−89% for amidite **23**, 90−92% for amidite **9**, and >99% for amidite **6** (with 10 min coupling time). For synthesis of the 16-mer **α-L-LNA-3**, the stepwise coupling yields obtained were 98−99% for amidite **23** (with 10 min coupling time). The different stepwise coupling yields obtained may be explained by slight variations in purity between the phosphoramidite samples. After standard deprotection and cleavage from the solid support by 32% aqueous ammonia (containing 1.5% LiCl in the case of the universal support) **α-L-LNA-1**, **α-L-LNA-2**, and **α-L-LNA-3** were purified by DMT-ON reversed-phase chromatography on disposable purification cartridges which includes detritylation. The composition of the α-L-LNAs were confirmed by MALDI-MS analysis and the purity (>90%) by capillary gel electrophoresis. MALDI-MS analysis: **α-L-LNA-1** $[M - H]^-$ 3405.8, calcd 3408.2; **α-L-LNA-2** $[M - H]^-$ 3636.2, calcd 3632.4; **α-L-LNA-3** $[M - H]^-$ 5025.4, calcd 5026.2.

Thermal Denaturation Studies. The thermal denaturation studies were carried out in a medium salt buffer solution (10 mM sodium phosphate, 100 mM sodium chloride, and 0.1 mM EDTA, pH 7.0). Concentrations of 1.5 mM of the two complementary strands were used, with the assumption of identical extinction coefficients for modified and unmodified oligonucleotides. The absorbance was monitored at 260 nm while the temperature was raised at a rate of 1 °C/min. The melting temperatures (T_m values) of the duplexes were determined as the maximum of the first derivatives of the melting curves obtained.

CD Experiments. The CD spectra were recorded on a Jasco J710 spectropolarimeter at room temperature in 0.5 cm cuvettes with the same oligonucleotide concentrations and hybridization buffer solution as those used for the thermal denaturation studies.

RNase H Experiments. The ribonucleotides **T2** and **T3** were radiolabeled (^{32}P) at the 5′-end with T_4 polynucleotide kinase (Gibco−BRL) and [γ-^{32}P]adenosine 5′-triphosphate (ATP) (4500 Ci/mmol, ICN) by standard procedures[39] and purified on NAP-5 columns (Pharmacia). Mixtures of the radiolabeled **T3** (2.5 pmol) and either the complementary deoxyribonucleotide **DNA-3** (5 pmol), **α-L-LNA-3** (5 pmol), or **LNA-3** (5 pmol), as well as a mixture of the radiolabeled **T2** (2.5 pmol) and either the complementary deoxyribonucleotide **DNA-1** (5 pmol), **α-L-LNA-1** (5 pmol), **LNA-1** (5 pmol), or **LNA-2** (5 pmol), were incubated at 37 °C for 15 min in a total volume of 25 μL (pH 7.5) containing 10 mM Tris-HCl, 25 mM KCl, and 0.5 mM $MgCl_2$ to allow the duplex to be formed. Cleavage reactions were started by the addition of 10 units of RNase H (*E. coli* ribonuclease H, Life Technologies) to the mixtures, while control tubes received no enzyme. Aliquots were taken at appropriate time intervals, mixed with an equal volume of stop mix (50 mM EDTA, 0.1% xylene cyanol FF, and 0.1% bromophenol blue in 90% formamide), and chilled in a dry ice/acetone bath. Samples were analyzed by denaturing 20% polyacrylamide gel electrophoresis (PAGE) containing urea (50%) with TBE buffer[39] at 1000 V for 1.25 h, followed by visualization by phosphorimaging (Packard Cyclone, OptiQuant software).

Stability toward 3′-Exonucleolytic Degradation. A solution of the oligonucleotides (~0.2 OD) in 2 mL of a buffer (0.1 M Tris-HCl, pH

(39) Maniatis, T.; Fritsch, E.; Sambrook, J. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NY, 1989.

8.6, 0.1 M NaCl, and 14 mM $MgCl_2$) was digested with 1.2 units of snake venom phosphodiesterase [30 μL of a solution in 5 mM Tris-HCl, pH 7.5, and 50% glycerol (by volume)] at 25 °C. The increase in absorbance at 260 nm during digestion was followed.

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Supporting Information Available: Description and experimental details of the individual synthetic steps of the preliminary conversion of furanose **26** into the guanine α-L-LNA nucleoside **35** and Scheme S1 illustrating this conversion; copies of the ^{13}C NMR spectra of compounds **7**, **8**, **11**, **14**, **16**, **19**, **20**−**22**, **26**−**32**, **34**, and **35**; copies of selected NOE spectra of compound **20**; and copies of the ^{31}P NMR spectra of compounds **9** and **23** (PDF). This material is available free of charge via the Internet at http:/pubs.acs.org.

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