Synthesis of Abasic Locked Nucleic Acid and Two *seco*-LNA Derivatives and Evaluation of Their Hybridization Properties Compared with Their More Flexible DNA Counterparts

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To investigate the structural basis of the unique hybridization properties of LNA (locked nucleic acid)[1] three novel LNA derivatives with modified carbohydrate parts were synthesized and evaluated with respect to duplex stabilities. The abasic LNA monomer[2] (**X**$^{\mathbf{L}}$, Figure 1) with the rigid carbohydrate moiety of LNA but no nucleobase attached showed no enhanced duplex stabilities compared to its more flexible abasic DNA counterpart (**X**, Figure 1). These results suggest that the exceptional hybridization properties of LNA primarily originate from improved intrastrand nucleobase stacking and not backbone preorganization. Two monocyclic *seco*-LNA derivatives, obtained by cleavage of the C1′−O4′ bond of an LNA monomer or complete removal of the O4′−furanose oxygen atom (**Z**$^{\mathbf{L}}$ and **dZ**$^{\mathbf{L}}$, respectively, Figure 1), were compared to their acyclic DNA counterpart[3] (**Z**, Figure 1). Even though they are more constrained than **Z**, the *seco*-LNA derivatives **Z**$^{\mathbf{L}}$ and **dZ**$^{\mathbf{L}}$ destabilize duplex formation even more than the flexible *seco*-DNA monomer **Z**.

Introduction

In the search for new high-affinity antisense oligoribonucleotide (ON) analogues, much of the recent research has been focused on the synthesis of conformationally restricted derivatives.[4,5] Several ONs containing bi- and tricyclic carbohydrate moieties have been synthesized, their hybridization properties have been studied,[6−14] and a number of these rigid analogues have displayed enhanced duplex stabilities compared with the corresponding unmodified ONs. The analogue termed LNA (locked nucleic acid, e.g., **T**$^{\mathbf{L}}$, Figure 1)[1,15−20] display most interesting properties: e.g., unprecedented thermal stabilities toward complementary DNA and RNA (ΔT_m/mod = 3−9 °C), stability toward 3′-exonucleolytic degradation, efficient automated oligomerization, and good aqueous solubility.[15−20]

The conformations of the furanose rings are decisive for the type of duplex formed between two complementary nucleic acid strands. The pentofuranose moieties in RNA normally exist in an N-type (north type) conformation leading to A-type duplexes, whereas an S-type (south type) conformation as generally seen for DNA leads to B-type duplexes. Studies on the interdependency between the dihedral phosphate backbone angle C4′−C3′−O3′−P and the furanose conformation likewise show that the furanose conformation leads to preorganization in the sugar−phosphate backbone.[21−23] This preorganization is also supported by recent NMR studies showing that unmodified nucleotides flanked by two LNA monomers (the rigid LNA monomer is restricted to an N-type (C3′-*endo*/^3E) conformation)[16,24] mainly exist in an N-type conformation, whereas all monomeric units of the un-

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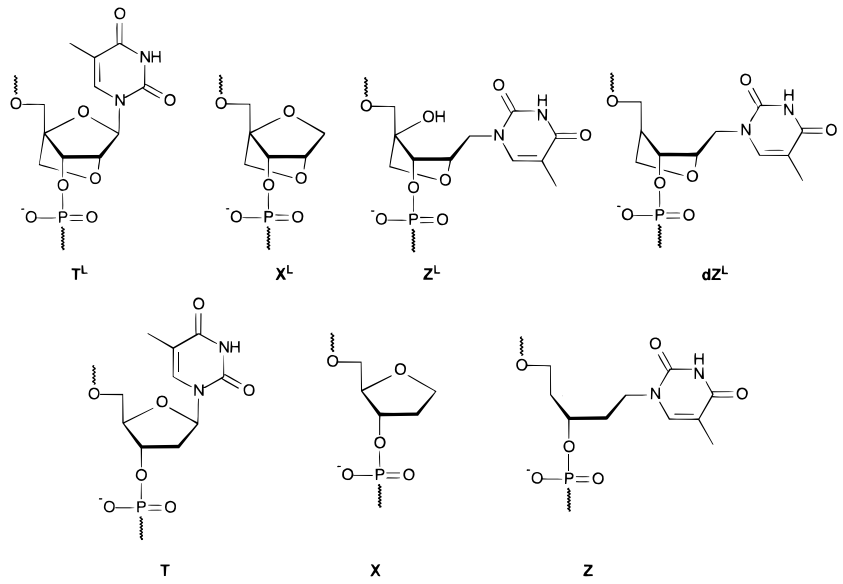


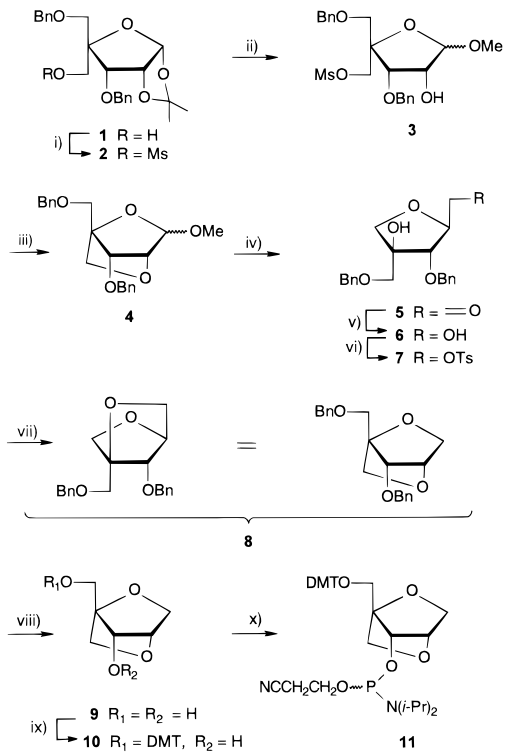
Figure 1. Structures of DNA and LNA monomers. $\mathbf{T^L}$ = thymine LNA monomer; $\mathbf{X^L}$ = abasic LNA monomer; $\mathbf{Z^L}$ = monocyclic thymine LNA monomer; $\mathbf{dZ^L}$ = deoxygenated monocyclic thymine LNA monomer; **T** = thymine DNA monomer; **X** = abasic DNA monomer; **Z** = acyclic thymine DNA monomer.

modified reference oligodeoxynucleotides showed the expected S-type conformation.[25] As further investigation of the importance of backbone preorganization on the stability of nucleic acid duplexes, we have synthesized and evaluated three novel LNA derivatives and compared their hybridization properties with those of their less conformationally restricted DNA counterparts. The abasic LNA monomer[2] $\mathbf{X^L}$ (Figure 1), which is anticipated to display the same restricted furanose and backbone conformations as the parent LNA monomers (e.g., as the monomer $\mathbf{T^L}$) with the exclusion of base−base stacking interactions, has been compared to the abasic DNA monomer **X**. Cleavage of the LNA C1′−O4′ bond or complete removal of the O4′ furanose oxygen atom has allowed the evaluation of the monocyclic *seco*-LNA monomers $\mathbf{Z^L}$ and $\mathbf{dZ^L}$, respectively. The synthesis of these was stimulated by the possibility of inducing some conformational restriction in the backbone, thereby potentially enhancing duplex stabilities compared to the totally flexible acyclic DNA monomer **Z**.[3] The rationale behind the design of LNA and the analogues introduced herein is the anticipation that more flexibility in a nucleic acid single strand results in a larger loss of entropy during duplex formation or less efficient nucleobase stacking which are believed to be the major reasons for the decreased duplex stabilities resulting from incorporation of acyclic nucleotides.[26,27] Furthermore, it is noteworthy that earlier studies have indicated that it is rotational restrictions in the sugar−phosphate backbone rather than nucleobase stacking that induces the helical-type structure reported for several single-stranded ONs.[28]

Results and Discussion

As previously reported, the 4-*C*-hydroxymethyl furanose **1**[29] (Scheme 1) was mesylated and transacetalized to give the anomeric mixture of methyl furanosides **3** (*via* **2**) which, by subsequent treatment with sodium hydride, gave the cyclized anomeric mixture of methyl furanosides **4**, and this, followed by hydrolysis (80% AcOH), afforded the monocyclic aldehyde **5**.[30] Reduction of this aldehyde

Scheme 1[a]



[a] Reagents and conditions: (i) MsCl, pyridine; (ii) 15% HCl, MeOH/H_2O (7:1), 85% (2 steps); (iii) NaH, DMF; (iv) 80% AcOH, (v) $NaBH_4$, MeOH, 86% (3 steps); (vi) TsCl, DMAP, CH_2Cl_2, 83%; (vii) NaH, DMF, 82%; (viii) H_2, 10% Pd/C, 80%; (ix) DMTCl, pyridine, 73%; (x) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 62%. DMT = 4,4′-dimethoxytrityl.

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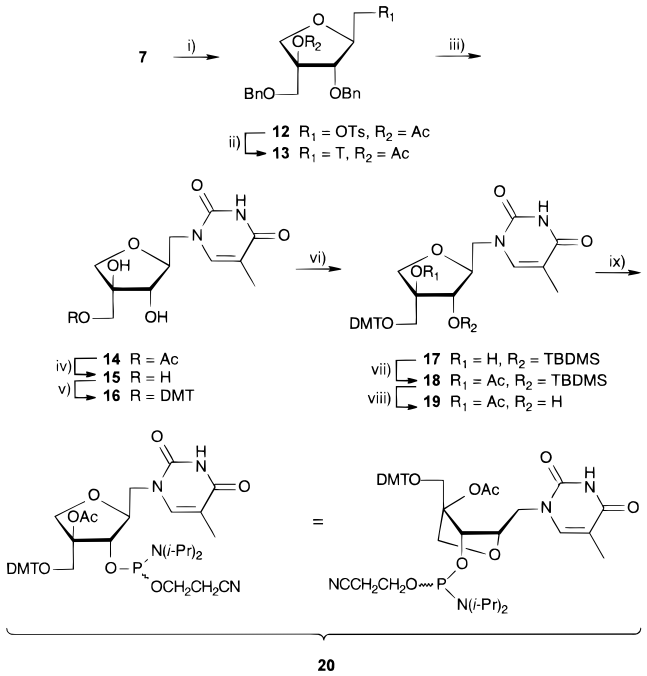
Scheme 2[a]



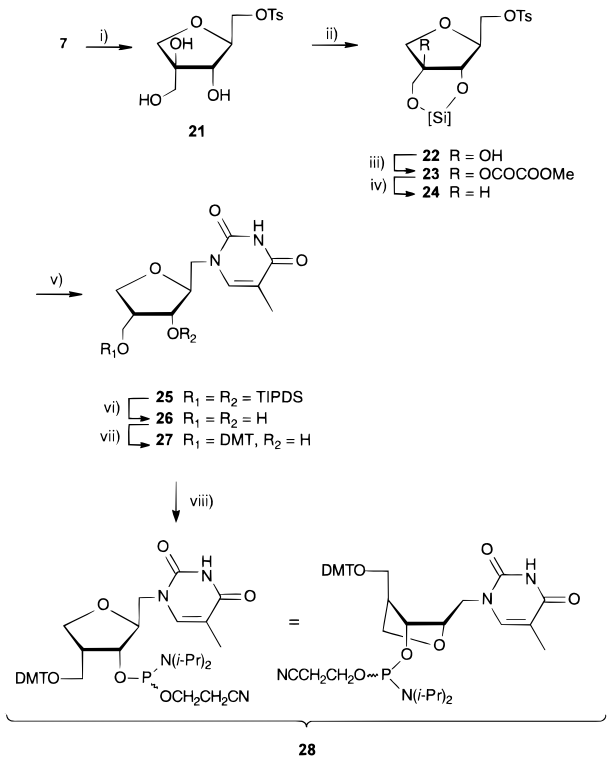
[a] Reagents and conditions: (i) Ac_2O, DMAP, pyridine, 84%; (ii) thymine, NaH, DMF, 52%; (iii) H_2, 20% $Pd(OH)_2/C$, EtOH, 87%; (iv) NaOMe, MeOH, 82%; (v) DMTCl, pyridine, 87%; (vi) TBDMSCl, imidazole, CH_2Cl_2, 69%; (vii) Ac_2O, DMAP, pyridine, 61%; (viii) TBAF, THF; (ix) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 67% (2 steps). DMT = 4,4′-dimethoxytrityl.

with $NaBH_4$ proceeded smoothly in 86% yield (from **3**) to give the diol **6** which afterward was tosylated selectively at the primary hydroxy group, affording the key intermediate **7** in 83% yield.[2] In the synthesis of the abasic LNA monomer $\mathbf{X^L}$, intermediate **7** was cyclized with NaH in DMF to give the bicyclic derivative **8** in 82% yield, which was debenzylated by hydrogenolysis to afford diol **9** in 80% yield. To prepare for automated oligonucleotide synthesis using the phosphoramidite approach,[31] derivative **9** was selectively DMT (4,4′-dimethoxytrityl) -protected at the primary hydroxy group to give **10** in 73% yield by reaction with DMTCl in pyridine, whereupon phosphitylation yielded the phosphoramidite building block **11**[2] in 62% yield (Scheme 1).

As a key step during the synthesis of the monocyclic *seco*-LNA thymine monomer $\mathbf{Z^L}$ (Scheme 2), introduction of the thymine moiety by nucleophilic substitution was envisioned. To prevent cyclization into derivative **8**, protection of the tertiary hydroxy group of intermediate **7** was needed. Initial attempts of *tert*-butyldimethyl silylation or benzoylation under various conditions failed, probably because of sterical hindrance. On the contrary, TMS-protection using TMSCl and imidazole in dichloromethane proceeded smoothly in 89% yield, but the O−Si bond was not stable enough for the concomitant substitution step (NaH and thymine in DMF at 120 °C), and the major product was in this case derivative **8**. Alternatively, after acetylation of **7** to give the fully protected derivative **12** in 84% yield using acetic anhydride and DMAP in pyridine, introduction of the nucleobase was successfully accomplished by reaction with NaH and thymine in DMF at 120 °C, affording the thymine derivative **13** in 52% yield. Unfortunately, debenzylation of **13** (H_2, 20% $Pd(OH)_2$, EtOH) induced quantitative migration of the acetyl group to the primary position, thereby furnishing **14** in 87% yield. This migration was indicated by changes in the δ ^{1}H of the neighboring methylene group and the δ ^{13}C of the quaternary carbon atom. It was furthermore verified from a ^{1}H NMR spectrum recorded in DMSO-d_6 where OH couplings clearly showed the free hydroxy groups to be secondary and tertiary. The migration was also supported by the fact that **14** could not be DMT protected, even with silver triflate as catalyst. To obtain a convenient phosphoramidite derivative for automated oligonucleotide synthesis, a number of protection group transformations were needed. Thus, **14** was deacetylated to give triol **15** in 82% yield using sodium methoxide in MeOH followed by selective DMT protection to furnish **16** in 87% yield (DMTCl in pyridine), selective silylation of the secondary hydroxy group in 69% yield (TBDMSCl and imidazole in dichloromethane) to give **17**, and acetylation in 61% yield as described above, thereby affording **18** with three orthogonal protecting groups. Eventually, desilylation to give **19** (TBAF in THF) and subsequent phosphitylation yielded the monocyclic *seco*-LNA phosphoramidite building block **20** (67% for two steps, Scheme 2).

In the first approach for synthesis of the O4′-deoxygenated (conventional nucleoside numbering used) monocyclic *seco*-LNA thymine phosphoramidite building block **28** (Scheme 3), direct deoxygenation of the 4-*O*-methoxalyl derivative of **7** using Bu_3SnH and AIBN in toluene[32]

Scheme 3[a]



[a] Reagents and conditions: (i) H_2, 20% $Pd(OH)_2/C$, EtOH, 86%; (ii) $TIPDSCl_2$, pyridine, 71%; (iii) methoxalyl chloride, DMAP, CH_2Cl_2, 74%; (iv) Bu_3SnH, AIBN, toluene, 84% (24:24a = 5:1); (v) thymine, NaH, DMF, 61%; (vi) TBAF, THF; (vii) DMTCl, 63% (2 steps); (viii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, 68%. [Si] = TIPDS = 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl; DMT = 4,4′-dimethoxytrityl.

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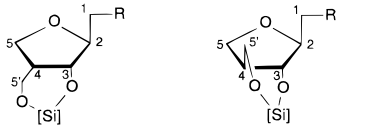

Figure 2. [Si] = 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl.

afforded an inseparable mixture of the two diastereoisomers resulting from reduction of the diastereotopic tertiary radical. To induce the desired diastereoselectivity via steric control, the 4-*O*-methoxalyl derivative **23** was prepared. Thus, compound **7** was debenzylated (H_2, 20% $Pd(OH)_2$, EtOH) to give triol **21** in 86% yield, which was then selectively protected with the bidentate reagent 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) in pyridine, affording the TIPDS-protected derivative **22** in 71% yield. The 4-*O*-methoxalyl derivative **23** was prepared by reaction of **22** with methoxalyl chloride and DMAP in dichloromethane. In one experiment, column chromatographic purification of the methoxalyl intermediate (74% yield) before the deoxygenation step (Bu_3SnH and AIBN in toluene) afforded a 5:1 mixture of O4-deoxygenetated diastereoisomers **24** and **24a** (Figure 2) in 84% yield as a result of diastereoselective reduction of the intermediary tertiary radical. Alternatively, direct deoxygenation of the crude 4-*O*-methoxalyl intermediate gave the same mixture in only 34% combined yield for the two steps. When the synthesis of the 4-*O*-methoxalyl derivative was repeated, this proved unstable during column chromatography (~50% decomposition to the alcohol **22** using CH_2Cl_2/MeOH mixtures as eluents). This could be minimized to ca. 10% decomposition (as judged from analytical TLC) by the addition of 0.5% pyridine to the eluent during column chromatography. Even though this solvent system did not afford totally pure **23**, the deoxygenation step again yielded a 5:1 mixture of the diastereomers **24** and **24a**, but this time in satisfactory 66% combined yield for the two steps. Subsequently, thymine was introduced to give derivative **25** in 61% yield by a substitution reaction on **24** (thymine and NaH in DMF), whereafter first desilylation (TBAF in THF to give diol **26**) and then selective DMT protection afforded **27** in 63% combined yield for two steps. Last, standard phosphitylation furnished the desired phosphoramidite derivative **28** in 68% yield (Scheme 3).

For conclusive structural verification, NOE difference spectroscopy experiments on the individual compounds of the two diastereoisomeric pairs **24**/**24a** and **25**/**25a** were performed (see Figure 2 for numbering used *in the discussion below*). Irradiation of H3 and H4 in **24** and **25** proved these two protons to be mutually close (NOEs of all four irradiations were 4−10%). For **24a**, irradiation of H2 and H4 showed the proximity of these two protons (NOEs of 2 and 4%, respectively), whereas irradiation of one of the H5′ methylene protons gave an NOE of 4% on H3. Irradiation of H4 in **25a** likewise showed an NOE on H2 of 4%. Altogether, these results unambiguously verify the configuration of **24** and **25** and thus also of **26**−**28**.

The modified 13- and 9-mer oligodeoxyribonucleotides (ODNs) and oligoribonucleotides (ONs) depicted in Table 1 were synthesized, and their hybridization to fully complementary ODN sequences 5′-*d*(TCGCATATCACTG) and 5′-*d*(GCATATCAC) was studied. Prolonged coupling times were used for amidites **11**, **20**, and **28** on an automated DNA synthesizer as outlined in details in the Experimental Section. The stepwise coupling yields for the modified phosphoramidites were >99% (6 min coupling time) for amidite **11**, >98% (15 min coupling time) for amidite **20**, and 95−99% (20 min coupling time) for amidite **28**. The stepwise coupling yields for the unmodified amidites and the amidites leading to incorporation of the $\mathbf{T^L}$ and **X** monomers were >99%. Surprisingly, the coupling with an unmodified RNA amidite (2′-*O*-TBDMS-protected RNA amidites were used) following an incorporation of amidite **28** proceeded in only 92−98% yield compared with yields of 95−99% following amidite **20** (20 min coupling time). Cleavage from the solid support and removal of protecting groups was accomplished using

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

entry	sequence	**Y**	T_m/°C	ΔT_m
1	5′-*d*(CAGTGA-**Y**-ATGCGA)	**T**	47.6	
2		$\mathbf{T^L}$	52.8	+5.2
3		**X**	27.2	−20.4
4		$\mathbf{X^L}$	26.7	−20.9
5		$\mathbf{Z^L}$	32.6	−15.0
6		$\mathbf{dZ^L}$	33.3	−14.3
7	5′-*d*(CAG-**Y**-GA-**Y**-A-**Y**-GCGA)	$\mathbf{T^L}$	58.6	+3.7
8		$\mathbf{Z^L}$	b	
9		$\mathbf{dZ^L}$	b	
10	5′-*r*(CAGUGA-**Y**-AUGCGA)	**U**	45.9	
11		**T**	44.8	−1.1
12		$\mathbf{T^L}$	52.8	+6.9
13		**X**	28.8	−17.1
14		$\mathbf{X^L}$	28.6	−17.3
15		$\mathbf{Z^L}$	32.5	−12.3
16		$\mathbf{dZ^L}$	35.9	−8.9
17	5′-*r*(CAG-**Y**-GA-**Y**-A-**Y**-GCGA)	**T**	43.3	−0.9
18		$\mathbf{T^L}$	66.0	+6.7
19		$\mathbf{Z^L}$	b	
20		$\mathbf{dZ^L}$	13.5	−10.4
21	5′-*d*(GTGA-**Y**-ATGC)	**T**	26.9	
22		$\mathbf{T^L}$	34.8	+7.9
23		**X**	b	
24		$\mathbf{X^L}$	b	
25		$\mathbf{Z^L}$	7.1	−19.8
26		$\mathbf{dZ^L}$	10.2	−16.7
27	5′-*r*(GUGA-**Y**-AUGC)	**U**	26.8	
28		**T**	25.8	−1.0
29		$\mathbf{T^L}$	37.9	+11.1
30		$\mathbf{X^L}$	~3	−23.3
31		$\mathbf{Z^L}$	9.2	−17.6
32		$\mathbf{dZ^L}$	13.2	−13.6
33	5′-*d*(T_7-**Y**-T_6) toward 5′-*d*(A_{14})	**T**	32	
34		$\mathbf{Z^L}$	20.1	−12.0
35		$\mathbf{dZ^L}$	19.3	−13.0
36	5′-*d*(T_7-**Y**-T_6) toward 5′-*r*(A_{14})	**T**	28	
37		$\mathbf{Z^L}$	20.5	−7.5
38		$\mathbf{dZ^L}$	20.5	−7.5

[a] T_m values measured as the maximum of the first derivative of the melting curve (A_{260} vs temperature) recorded in medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 μM concentrations of the two complementary strands (assuming identical extinction coefficients for T and all thymine-containing monomeric nucleotides and no extinction for the abasic monomers). ΔT_m = change in T_m value calculated *per modification*. A = adenosine monomer, C = cytidine monomer, G = guanosine monomer, U = uridine monomer, T = thymidine monomer, $\mathbf{T^L}$, **X**, $\mathbf{X^L}$, **Z**, $\mathbf{Z^L}$, $\mathbf{dZ^L}$; see Figure 1. Oligo-2′-deoxynucleotide sequences are depicted as *d*(sequence) and oligoribonucleotide sequences as *r*(sequence). [b] No T_m was detected.

(32) Dolan, S. C.; Macmillan: J. *J. Chem. Soc., Chem. Commun.* **1985**, 1588.

concentrated aqueous ammonia (55 °C for 16 h) except for sequences containing monomer $\mathbf{Z^L}$ or RNA monomers where aq methylamine (40% (w/w); 10 min, 55 °C) was used (for further details see the Experimental Section). Capillary gel electrophoresis of all synthesized sequences showed the purity as >90%, and the compositions were verified by MALDI-MS analysis. When the standard conditions (concentrated aq ammonia; 55 °C for 16 h) were used during deprotection and cleavage from the solid support for sequences containing monomer $\mathbf{Z^L}$, the acetyl group at the 4′-*O* position of monomer $\mathbf{Z^L}$ was removed which could lead to intramolecular attack of the resulting free tertiary hydroxy group on the phosphorus atom and thereby to strand cleavage. This is a plausible explanation for the generation of minor byproducts of the predicted masses detected by MALDI-MS under these conditions. As described above, all sequences containing $\mathbf{Z^L}$ were cleaved and deprotected using methylamine instead which afforded complete deprotection without any detectable strand cleavage. For each sequence, the melting temperature (T_m value) and the change in T_m value per modification compared with the unmodified reference (ΔT_m) were determined[33] (Table 1).

Incorporation of a single LNA monomer $\mathbf{T^L}$ profoundly increases the thermal stability (ΔT_m values of 5.2−11.1 °C (entries 2, 12, 22, and 29 in Table 1). In accordance with previous reports,[34] we observed a detrimental effect on the thermal stability upon incorporation of the abasic DNA monomer **X** (entries 3, 13, and 23, ΔT_m values < −17 °C). The T_m values obtained for the abasic LNA monomer $\mathbf{X^L}$ (entries 4, 14, 24, and 30) indicate that no stabilization results from exchange of the abasic DNA monomer **X** with the abasic LNA monomer $\mathbf{X^L}$. This is in strong contrast to the results obtained by incorporation of one LNA monomer $\mathbf{T^L}$ instead of a thymidine monomer and indicates that the direct effect of backbone preorganization is only a minor contributor to the increased duplex stabilities of LNA. It is during these considerations anticipated that the conformations of the furanose−phosphate moieties of $\mathbf{X^L}$ and $\mathbf{T^L}$ are identical and that the conformational effect of a nucleobase is negligible in an LNA monomer. We believe that the strongly constrained furanose moieties of $\mathbf{X^L}$ and $\mathbf{T^L}$ warrant these assumptions. These results support the recent NMR investigations leading to the conclusion that it is mainly improved nucleobase stacking which confers preorganization to an LNA and thus the exceptional stability of duplexes involving LNA.[25] It should be noted that the effect of this favorable nucleobase stacking could be of both enthalpic as well as entropic origin and that we have earlier reported the hybridization of a fully modified LNA to be enthalpically driven and that of a partly modified LNA to be entropically driven.[19]

The monocyclic *seco*-LNA monomers $\mathbf{Z^L}$ and $\mathbf{dZ^L}$ were incorporated in the same mixed sequences as $\mathbf{X^L}$ as well as in a homothymine sequence for direct comparison with the previously reported results for the acyclic DNA monomer **Z**.[3] It was reported that monomer **Z** when incorporated in the middle of an oligothymidylate 13-mer induced a decrease in the T_m value of 8.1 °C toward complementary DNA and a much less pronounced destabilizing effect (ΔT_m = −0.3 °C) when incorporated in the exterior of the T_{13} strand.[3] By the presence of one additional tetrahydrofuran ring, the less flexible *seco*-LNA counterparts $\mathbf{Z^L}$ and $\mathbf{dZ^L}$ could induce enhanced binding of complementary strands if favorable conformations of the tetrahydrofuran rings are being adopted. In parent LNA, this ring is locked in an envelope conformation with the C3 atom (numbering as in Figure 2) out of the plane created by the remaining four ring atoms. As judged by the variations in coupling constants between the H2 and H3 atoms (numbering as in Figure 2) of **15**−**18**, **25**, and **26** (*J* values of 2.7−8.1 Hz), it is clear that there is no preference for the apparently ideal LNA-type conformation of this ring. Thus, the possibility of increased duplex stabilities (compared to the flexible DNA monomer **Z**) for the monocyclic monomers $\mathbf{Z^L}$ and $\mathbf{dZ^L}$ seems very much counteracted (see below) by overall unfavorable conformations leading possibly to detrimental sterical interactions during duplex formation.[35] Experimentally, very large decreases in melting temperatures (ΔT_m = −9 to −17 °C for $\mathbf{dZ^L}$, entries 6, 9, 16, 20, 26, and 32; ΔT_m = −12 to −17 °C for $\mathbf{Z^L}$, entries 5, 8, 15, 19, 25, and 31) were obtained. For the 14-mer oligothymidylate sequence (entries 33−38), similar ΔT_m values were observed (toward both complementary DNA and RNA). Summarizing, the tetrahydrofuran ring of monomers $\mathbf{Z^L}$ and $\mathbf{dZ^L}$ seems to force these nucleotides into conformations unfavorable for duplex formation possibly by restricting the number of attainable conformations.

Conclusion

The results for the abasic monomers **X** and $\mathbf{X^L}$ compared with those of **T** and $\mathbf{T^L}$ emphasize the importance of the nucleobase as a mediator of conformational changes leading to enhanced duplex stabilities. The structural difference for both pairs examined ($\mathbf{T^L}$ vs T and $\mathbf{X^L}$ vs **X**) is a methylene bridge between the O2′ and the C4′ atoms thereby indicating that conformational restrictions of the pentofuranose−phosphate backbone alone are not sufficient to induce an effect on the thermal stability. Despite the fact that the monocyclic *seco*-LNA derivatives $\mathbf{Z^L}$ and $\mathbf{dZ^L}$ are more constrained than the completely flexible acyclic DNA monomer **Z**,[3] they destabilize duplexes even more than **Z**. Even though the results obtained with the abasic monomers question the importance of backbone preorganization for entropically favored duplex formation, this destabilization is mainly believed to be caused by the tetrahydrofuran ring forcing the two monomers $\mathbf{Z^L}$ and $\mathbf{dZ^L}$ into conformations unfavorable for duplex formation.

Experimental Section

General. All reagents were obtained from commercial suppliers and were used without further purification. Petroleum ether of distillation range 60−80° was used. The silica gel (0.040−0.063 mm) used for column chromatography was purchased from Merck. After column chromatography, fractions containing product were pooled, evaporated under reduced pressure, and dried under vacuum to give the product. All ^{1}H NMR spectra were recorded at 400 MHz, all ^{13}C NMR spectra were recorded at 100.6 MHz (apart from two which were recorded at 75.5 MHz), and the three ^{31}P spectra were recorded at 121.5 MHz. Chemical shifts are reported in ppm

(33) T_m values were obtained as reported earlier. See ref 16.

(34) Millican, T. A.; Chauncey, M. A.; Cutbush, S. D.; Eaton, M. A. W.; Gunning, J.; Mann, J.; Mock, G. A.; Neidle, S.; Patel, T. P. *Nucleic Acids Res.* **1984**, *12*, 7435.

(35) Reduced binding affinities have also been observed for other ON analogues containing a flexible nucleobase on a rigified phosphordiester backbone. (a) Marangoni, M.; Van Aershot, A.; Augustyns, P.; Rozenski, J.; Herdewijn, P. *Nucleic Acids Res.* **1997**, *25*, 3034. (b) Epple, C.; Leumann, C. *Chem. Biol.* **1998**, *5*, 209.

relative to tetramethylsilane as internal standard for ^{1}H and ^{13}C and relative to 85% H_3PO_4 as external standard for ^{31}P. Assignments of NMR spectra are based on 2D spectra when given and follows standard carbohydrate/nucleoside nomenclature for easy comparison (i.e., the carbon atom next to the nucleobase is assigned C1′ etc.) even though the systematic compound names are given according to IUPAC 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.

3,5-Di-*O*-benzyl-1,2-*O*-isopropylidene-4-*C*-methanesulfonyloxymethyl-α-D-ribofuranose (2). Furanose **1**[29] (12.357 g, 30.9 mmol) was dissolved in anhydrous pyridine (25 mL) at 0 °C under a N_2 atmosphere, MsCl (5.00 mL, 64.3 mmol) was added, and the mixture was stirred for 20 min at room temperature. Half saturated aqueous $NaHCO_3$ (200 mL) was added, the mixture was extracted with EtOAc, and the combined organic phase was successively washed with saturated aqueous $NaHCO_3$ and H_2O. The combined organic phase was evaporated to dryness under reduced pressure and coevaporated with toluene (20 mL) to give a crude product (colorless oil, tentatively assigned as furanose **2**) which was used in the next step without purification. R_f (2% MeOH in CH_2Cl_2, (v/v)) 0.70.

Methyl 3,5-Di-*O*-benzyl-4-*C*-methylsulfonyloxymethyl-α,β-D-ribofuranoside (3).[30] Crude **2** was stirred in a mixture of H_2O (30 mL) and 15% HCl in MeOH (300 mL, w/w) for 10 h whereupon the mixture was neutralized with $NaHCO_3$ (s). H_2O (200 mL) was added, the mixture was extracted with EtOAc, and the combined organic phase was washed with H_2O and evaporated to dryness under reduced pressure. The residue was coevaporated with acetonitrile (100 mL), and the residue was subjected to silica gel column chromatography (14.5 × 5.5 cm) eluting with a gradient of 30−40% EtOAc in petroleum ether (v/v) to give furanosides **3** (11.896 g, 85.2% from **1**, anomeric ratio = 1:4) as a colorless oil which was used in the next step without further purification. R_f (EtOAc/petroleum ether, 1:1 (v/v)) 0.37; ^{13}C NMR ($CDCl_3$) δ 137.48, 136.74, 128.43, 128.37, 128.32, 128.29, 128.08, 127.93, 127.89, 127.82, 127.70, 127.60, 107.35, 103.18, 84.49, 83.01, 81.76, 78.75, 74.89, 73.93, 73.59, 73.23, 72.43, 71.98, 70.73, 70.42, 70.08, 55.78, 54.84, 37.10, 37.00.

(1*S*,3*RS*,4*R*,7*S*)-7-Benzyloxy-1-benzyloxymethyl-3-methoxy-2,5-dioxabicyclo[2.2.1]heptane (4).[30] Furanosides **3** (9.188 g, 13.3 mmol) were dissolved in anhydrous DMF (50 mL) under a N_2 atmosphere at 0 °C whereafter NaH (60% (w/w), 1.576 g, 39.4 mmol) was added over 5 min. The mixture was stirred at room temperature for 2 h, H_2O (200 mL) was added, and the suspension was extracted with EtOAc and washed with saturated aqueous $NaHCO_3$ and H_2O. The aqueous phase was extracted with EtOAc, and the combined organic phase was evaporated to dryness under reduced pressure to give a crude product (colorless oil, tentatively assigned as **4**) which was used without purification in the next step. R_f (EtOAc/petroleum ether, 3:7 (v/v)) 0.37, 0.10 (two epimers).

(2*R*,3*S*,4*S*)-3-Benzyloxy-4-benzyloxymethyl-2-formyl-4-hydroxytetrahydrofurfuran (5).[30] Crude **4** was stirred in 80% AcOH (100 mL) at 50 °C for 3.5 h whereupon the mixture was evaporated to dryness under reduced pressure and coevaporated with anhydrous toluene (2 × 20 mL) to give a crude product (colorless oil, tentatively assigned as **5**) which was used without purification in the next step. R_f (EtOAc/petroleum ether, 1:1 (v/v)) 0.42.

(2*S*,3*S*,4*S*)-3-Benzyloxy-4-benzyloxymethyl-2-hydroxymethyl-4-hydroxytetrahydrofuran (6). Crude **5** was dissolved in MeOH (100 mL), and $NaBH_4$ (2.138 g, 56.5 mmol) was added over 5 min. After being stirred for 20 min at room temperature, the reaction was quenched by the addition of 80% AcOH (10 mL), and the mixture was evaporated to dryness under reduced pressure and coevaporated with anhydrous toluene (20 mL). The residue was purified by silica gel column chromatography (15 × 5.8 cm) eluting with a gradient of 20−80% EtOAc in petroleum ether (v/v) to give furan **5** (5.776 g, 86.2% from **3**) as a colorless oil. R_f (EtOAc/petroleum ether, 3:1 (v/v)) 0.31; ^{1}H NMR ($CDCl_3$) δ 7.34−7.25 (10H, m, Bn), 4.58 (4H, d, *J* 10.8 Hz, Bn), 4.05 (1H, m, H2), 3.87−3.74 (5H, m), 3.60 (2H, m) (H3, H5, H3′, H5′), 2.4 (2H, bs, 2 × OH); ^{13}C NMR ($CDCl_3$) δ 137.54, 137.48, 128.32, 128.30, 127.74, 127.71, 127.67, 127.54 (Bn), 85.21, 84.83 (C2, C3), 81.50 (C4), 75.07 (C5), 73.68, 72.34 (Bn), 69.56 (C5′), 62.84 (C1).

(2*S*,3*S*,4*S*)-3-Benzyloxy-4-benzyloxymethyl-2-(*p*-toluenesulfonyl)oxymethyl-4-hydroxytetrahydrofuran (7). Compound **6** (6.994 g, 20.3 mmol) was dissolved in anhydrous CH_2Cl_2 (100 mL) at 0 °C under a N_2 atmosphere, DMAP (6.262 g, 51.26 mmol) followed by TsCl (7.688 g, 40.33 mmol) were added, and the mixture was stirred for 10 h at room temperature. After evaporation to dryness under reduced pressure, the residue was redissolved in EtOAc (250 mL), washed successively with saturated aqueous $NaHCO_3$ and H_2O, and the organic phase was evaporated to dryness under reduced pressure. The residue was subjected to column chromatography on silica gel (15 × 5.5 cm) using 1% MeOH in CH_2Cl_2 (v/v) as the eluent to give furan **7** (8.387 g, 82.8%) as a white solid material after coevaporation with *n*-hexane (100 mL). R_f (EtOAc/petroleum ether, 1:1 (v/v)) 0.55; FAB-MS *m*/*z* 499 [M + H]$^+$; ^{1}H NMR ($CDCl_3$) δ 7.75 (2H, d, *J* 8.2 Hz, Ar), 7.36−7.24 (12H, m, Bn, Ar), 4.56 (1H, d, *J* 11.8 Hz, Bn), 4.55 (1H, d, *J* 11.9 Hz, Bn), 4.53 (1H, d, *J* 11.8 Hz, Bn), 4.50 (1H, d, *J* 11.9 Hz, Bn), 4.14−4.09 (3H, m, H2, H1), 3.78 (1H, d, *J* 9.3 Hz, H5′), 3.76 (1H, s, H3), 3.76−3.74 (2H, m, H5), 3.49 (1H, d, *J* 9.3 Hz, H5′), 2.41 (3H, s, $ArCH_3$), 2.3 (1H, bs, OH); ^{13}C NMR ($CDCl_3$) δ 144.74, (Ar), 137.32, 137.26 (Bn), 132.68 (Ar), 129.69 (Ar), 128.38, 128.33, 127.91, 127.86, 127.77, 127.69, 127.67 (Bn, Ar), 84.28 (C3), 81.49 (C4), 81.33 (C2), 75.00 (C5), 73.65, 71.91 (Bn), 69.31, 69.19 (C5′, C1), 21.51 ($ArCH_3$). Anal. Calcd for ($C_{27}H_{30}O_7S$): C, 65.0; H, 6.1; S, 6.4. Found: C, 64.9; H, 6.0; S, 6.5.

(1*S*,4*S*,7*S*)-7-Benzyloxy-1-benzyloxymethyl-2,5-dioxabicyclo[2.2.1]heptane (8). Compound **7** (950 mg, 1.92 mmol) was dissolved in anhydrous DMF (8 mL) at 0 °C under a N_2 atmosphere, and NaH (134 mg, 60% (w/w), 3.35 mmol) was added. The mixture was stirred at room temperature for 1.5 h, H_2O (40 mL) was added, and the suspension was extracted with EtOAc. The combined organic phase was washed successively with saturated aqueous $NaHCO_3$ and H_2O. The combined aqueous phase was extracted with EtOAc, and the combined organic phase was evaporated to dryness under reduced pressure and coevaporated with anhydrous toluene (6 mL). The residue was purified by silica gel column chromatography (8.5 × 4.8 cm) using EtOAc/petroleum ether (3:7, v/v) as the eluent to give derivative **8** (516 mg, 82.0%) as a colorless oil. R_f (EtOAc/petroleum ether, 1:1 (v/v)) 0.44; FAB-MS *m*/*z* 327 [M + H]$^+$; ^{1}H NMR ($CDCl_3$) δ 7.37−7.26 (10H, m, Bn), 4.78 (1H, d, *J* 11.7 Hz, Bn), 4.63 (1H, d, *J* 12.9 Hz, Bn), 4.59 (1H, d, *J* 12.3 Hz, Bn), 4.58 (1H, d, *J* 12.0 Hz, Bn), 4.30 (1H, s, H3), 4.07−3.93 (5H, m), 3.73 (2H, s) (H1, H2, H5, H5′); ^{13}C NMR ($CDCl_3$) δ 137.79, 137.58, 128.40, 128.36, 127.83, 127.67, 127.58 (Bn), 85.74 (C4), 79.99, 76.45, 74.25, 73.75, 72.59, 71.94 (C1, C2, C3, C5′, Bn), 66.38 (C5).

(1*S*,4*S*,7*S*)-7-Hydroxy-1-hydroxymethyl-2,5-dioxabicyclo[2.2.1]heptane (9). To a solution of 10% Pd/C (0.05 g) in anhydrous EtOH (6 mL) was added **8** (516 mg, 1.58 mmol) dissolved in anhydrous EtOH (6 mL) under a N_2 atmosphere. The solution was degassed with H_2 and stirred under a H_2 atmosphere for 3.5 h. The mixture was evaporated to dryness under reduced pressure, and the residue was subjected to silica gel column chromatography (6 × 2.8 cm) using 10% MeOH in CH_2Cl_2 (v/v) as the eluent to give **9** (184 mg, 79.7%) as a white solid material. R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.22; FAB-MS *m*/*z* 147 [M + H]$^+$; ^{1}H NMR (CD_3OD) δ 4.04 (1H, s, H2), 4.01 (1H, s, H3), 3.89 (1H, d, *J* 7.9 Hz, H5′), 3.85 (1H, dt, *J* 8.0, 0.5 Hz, H1), 3.75 (1H, d, *J* 7.9 Hz, H5′), 3.74 (1H, d, *J* 8.2 Hz, H1), 3.70 (1H, d, *J* 15.2 Hz, H5), 3.67 (1H, d, *J* 15.2 Hz, H5); ^{13}C NMR (CD_3OD) δ 86.28 (C4), 78.72 (C2), 72.54 (C3), 72.38 (C5′), 71.21 (C1), 57.68 (C5).

(1*R*,4*S*,7*S*)-1-(4,4′-Dimethoxytrityl)oxymethyl-7-hydroxy-2,5-dioxabicyclo[2.2.1]heptane (10). Compound **9** (160 mg, 1.09 mmol) was dissolved in anhydrous pyridine (3

mL) at 0 °C under a N_2 atmosphere, DMTCl (570 mg, 1.68 mmol) was added, and the mixture was stirred at room temperature for 2.5 h. Sat. aq. $NaHCO_3$ (25 mL) was added, the mixture was extracted with EtOAc, and the combined organic phase was washed with H_2O. The organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with anhydrous toluene (2 × 10 mL). The residue was purified thrice by column chromatography using (a) 97.5:2.0:0.5 CH_2Cl_2/MeOH/pyridine (v/v/v), (b) 98.5:1.0:0.5 CH_2Cl_2/MeOH/pyridine (v/v/v), and (c) 70:30:0.5 petroleum ether/EtOAc/pyridine (v/v/v) as the eluents, thereby affording **10** (359 mg, 73.1%) as a white foam after coevaporation with anhydrous acetonitrile (3 × 10 mL). R_f (5% MeOH in CH_2Cl_2 (v/v)) 0.41; FAB-MS *m/z* 448 $[M]^+$; ^{1}H NMR (CD_3OD) δ 7.45−7.43 (2H, m, DMT), 7.33−7.23 (6H, m, DMT), 7.19−7.17 (1H, m, DMT), 6.84−6.81 (4H, m, DMT), 4.12 (1H, s, H2), 4.06 (1H, s, H3), 4.05 (1H, d, *J* 8.1 Hz, H1/H5′), 3.97 (2H, d, *J* 7.0 Hz, H1/H5′), 3.88 (1H, d, *J* 8.5 Hz, H1/H5′), 3.41 (1H, d, *J* 10.4 Hz, H5), 3.31 (1H, d, *J* 10.6 Hz, H5), 3.75 (6H, s, OCH_3); ^{13}C NMR (CD_3OD) δ 160.07, 146.34, 137.23, 137.06, 131.32, 131.28, 129.31, 128.70, 127.73, 114.05 (DMT), 87.34, 87.27 (CAr_2Ph, C-1), 80.49 (C4), 75.13, 74.90 (C3/C6, C7), 73.21(C3/C6), 62.37 (C2′), 55.70 (OCH_3).

(1*R*,4*S*,7*S*)-1-(4,4′-Dimethoxytrityl)oxymethyl-7-(2-cyanoethoxy(diisopropylamino)phosphinoxy)-2,5-dioxabicyclo[2.2.1]heptane (11). Derivative **10** (287 mg, 0.638 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (2 mL) and *N*,*N*-diisopropylethylamine (1 mL). 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0. 25 mL, 1.12 mmol) was slowly added under a N_2 atmosphere, and the resulting mixture was stirred at room temperature for 1 h whereupon MeOH (20 mL) and EtOAc (50 mL) were added. The mixture was washed with saturated aqueous $NaHCO_3$, and the organic phase was evaporated to dryness under reduced pressure. The residue was subjected to silica gel chromatography twice with (a) 70:30:0.5 petroleum ether/EtOAc/pyridine (v/v/v) and (b) 75:25:0.5 petroleum ether/EtOAc/pyridine (v/v) as the eluents to give **11** (258 mg, 62.3%) as a white foam after coevaporation with anhydrous acetonitrile (6 × 10 mL). R_f (1:1 EtOAc/petroleum ether (v/v)) 0.65, 0.57 (2 diastereomers); FAB-MS *m/z* 649 $[M + H]^+$; ^{31}P NMR ($CDCl_3$) δ 148.41, 148.32.

(2*S*,3*S*,4*S*)-4-Acetoxy-3-benzyloxy-4-benzyloxymethyl-2-((*p*-toluenesulfonyl)oxymethyl)tetrahydrofuran (12). Compound **7** (3.442 g, 6.90 mmol) was dissolved in anhydrous pyridine (20 mL) under a N_2 atmosphere whereafter DMAP (3.292 g, 26.9 mmol) and Ac_2O (1.5 mL, 15.9 mmol) were added. The reaction mixture was stirred at room temperature for 2 h, H_2O (200 mL) was added, and the suspension was extracted with EtOAc. The combined organic phase was washed successively with saturated aqueous $NaHCO_3$ and H_2O, evaporated to dryness under reduced pressure, and coevaporated with anhydrous toluene (3 × 10 mL). The residue was purified by silica gel column chromatography (12.3 × 5.5 cm) with EtOAc/petroleum ether (1:4, v/v) as the eluent yielding **12** (3.142 g, 84.2%) as a colorless oil after coevaporation with *n*-hexane (3 × 15 mL). R_f (1:1 EtOAc/petroleum ether (v/v)) 0.73; FAB-MS *m/z* 541 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.75 (2H, d, *J* 8.4 Hz, Ar), 7.32−7.22 (14H, m, Ar, 2 × Bn), 4.67 (1H, d, *J* 11.5 Hz, Bn), 4.51 (1H, d, *J* 11.5 Hz, Bn), 4.49 (2H, d, *J* 11.8 Hz, Bn), 4.25 (1H, d, *J* 10.6 Hz, H5), 4.10 (1H, d, *J* 10.1 Hz, H5′), 4.08 (1H, s, H3), 4.01 (2H, m, H1), 3.96 (1H, m, H2), 3.94 (1H, d, *J* 10.1 Hz, H5′), 3.85 (1H, d, *J* 10.6 Hz, H5), 2.42 (3H, s, $ArCH_3$), 2.01 (3H, s, $COCH_3$); ^{13}C NMR ($CDCl_3$) δ 170.18 (C═O), 144.78 (Ar), 137.71, 137.26 (Bn), 132.61 (Ar), 129.68, 128.31, 128.23, 127.87, 127.77, 127.58, 127.43 (Ar, Bn), 89.05 (C4), 83.17 (C3), 81.07 (C2), 73.70, 73.46, 73.07 (2 × Bn, C5), 68.52 (C1), 66.49 (C5′), 21.64, 21.51 ($ArCH_3$, $COCH_3$).

(2*S*,3*S*,4*S*)-4-Acetoxy-3-benzyloxy-4-benzyloxymethyl-2-(thymin-1-ylmethyl)tetrahydrofuran (13). Thymine (1.539 g, 12.2 mmol) was dissolved under a N_2 atmosphere in anhydrous DMF (40 mL) at 120 °C. NaH (495 mg, 60% (w/w), 12.4 mmol) was added, and the suspension was stirred for 10 min whereupon **12** (3.142 g, 5.81 mmol) dissolved in anhydrous DMF (25 mL) was slowly added. After 4 h, H_2O (150 mL) was added, and the mixture was extracted with EtOAc. The combined organic phase was washed successively with saturated aqueous $NaHCO_3$ and H_2O, evaporated to dryness under reduced pressure, and coevaporated with anhydrous toluene (2 × 15 mL). The residue was purified by silica gel column chromatography (9.8 × 5.5 cm) eluting with a gradient of 30%−70% EtOAc in petroleum ether (v/v) to give **13** (1.484 g, 51.6%) as a white foam after coevaporation with *n*-hexane (3 × 20 mL). R_f (1:1 EtOAc/petroleum ether (v/v)) 0.18; FAB-MS *m/z* 495 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.81 (1H, bs, NH), 7.34−7.26 (10H, m, Bn), 7.04 (1H, d, *J* 1.3 Hz, H6), 4.74 (1H, d, *J* 11.7 Hz, Bn), 4.62 (1H, d, *J* 11.5 Hz, Bn), 4.53 (1H, d, *J* 11.9 Hz, Bn), 4.49 (1H, d, *J* 11.9 Hz, Bn), 4.32 (1H, d, *J* 10.8 Hz, H5′), 4.10 (1H, d, *J* 10.1 Hz, H5″), 4.00−3.94 (4H, m, H1′, H2′, H3′, H5″), 3.84 (1H, d, *J* 10.8 Hz, H5′), 3.74 (1H, m, H1′), 2.04 (3H, s, CH_3CO), 1.89 (3H, d, *J* 1.1 Hz, CH_3);^{13}C NMR ($CDCl_3$) δ 170.18 (C═O), 163.92 (C4), 150.82 (C2), 141.19 (C6), 137.65, 137.36, 128.33, 128.25, 127.89, 127.83, 127.62, 127.51 (Bn), 110.02 (C5), 89.26 (C4′), 84.23 (C2′), 82.07 (C3′), 73.71, 73.51, 73.29 (Bn, C5′), 66.55 (C5″), 48.90 (C1′), 21.73 (CH_3CO), 12.19 (CH_3).

(2*S*,3*S*,4*R*)-4-Acetoxymethyl-3,4-dihydroxy-2-(thymin-1-ylmethyl)tetrahydrofuran (14). Compound **13** (1.897 g, 3.84 mmol) was dissolved in anhydrous EtOH (30 mL), 20% Pd-$(OH)_2$/C (0.08 g) was added, and the mixture was degassed with H_2 under reduced pressure. After being stirred at room temperature under a H_2 atmosphere for 36 h, the mixture was evaporated to dryness under reduced pressure, and the residue was subjected to column chromatography on silica gel (8.0 × 5.5 cm) eluting with a gradient of 5−7% MeOH in CH_2Cl_2 (v/v) to give **14** (1.054 g, 87.4%) as a white foam after coevaporation with *n*-hexane (2 × 10 mL). R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.24; FAB-MS *m/z* 315 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.43 (1H, q, *J* 1.1 Hz, H6), 4.33 (1H, d, *J* 11.7 Hz, H5″), 4.25 (1H, d, *J* 11.5 Hz, H5″), 4.14 (1H, dd, *J* 3.8, 13.8 Hz, H1′), 4.05 (1H, m, H2′), 3.95−3.88 (4H, m, H1′, H3′, H5′), 2.12 (3H, s, CH_3CO), 1.90 (3H, d, *J* 1.3 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.97 (CH_3CO), 165.00 (C4), 151.09 (C2), 142.32 (C6), 108.50 (C5), 84.36 (C2′), 79.93 (C4′), 78.44 (C3′), 74.11 (C5′), 64.78 (C5″), 49.51 (C1′), 18.83 (CH_3CO), 10.31 (CH_3).

(2*S*,3*S*,4*S*)-3,4-Dihydroxy-4-hydroxymethyl-2-((thymin-1-yl)methyl)tetrahydrofuran (15). Compound **14** (1.037 g, 3.30 mmol) was dissolved in anhydrous MeOH (20 mL) at 0 °C under a N_2 atmosphere, NaOMe (541 mg, 10.0 mmol) was added, and the mixture was stirred for 50 min whereupon 80% aq AcOH (2 mL) was added. The mixture was evaporated to dryness under reduced pressure, coevaporated with anhydrous toluene (10 mL), and the residue was purified by silica gel column chromatography (5.5 × 5.5 cm) using 10% MeOH in CH_2Cl_2 (v/v) as the eluent, affording **15** (716 mg, 81.6%) as a white foam. R_f (20% MeOH in CH_2Cl_2 (v/v)) 0.32; FAB-MS *m/z* 273 $[M + H]^+$; ^{1}H NMR (CD_3OD) δ 7.44 (1H, d, *J* 1.2 Hz, H6), 4.15 (1H, dd, *J* 3.7, 13.7 Hz, H1′), 4.03 (1H, dt, *J* 3.7, 8.1 Hz, H2′), 3.94−3.84 (4H, m, H1′, H3′, H5′), 3.79 (1H, d, *J* 11.5 Hz, H5″), 3.71 (1H, d, *J* 11.5 Hz, H5″), 1.90 (3H, d, *J* 1.3 Hz, CH_3); ^{13}C NMR (CD_3OD) δ 165.03 (C4), 151.10 (C2), 142.34 (C6), 108.51 (C5), 84.19 (C2′), 81.37 (C4′), 78.72 (C3′), 74.18 (C5′), 62.14 (C5″), 49.56 (C1′), 10.30 (CH_3).

(2*S*,3*S*,4*R*)-3,4-Dihydroxy-4-(4,4′-dimethoxytrityl)oxymethyl-2-((thymin-1-yl)methyl)tetrahydrofuran (16). Compound **15** (706 mg, 2.59 mmol) dissolved in anhydrous pyridine (20 mL) was concentrated under reduced pressure to half volume and cooled to 0 °C under a N_2 atmosphere. 4,4′-Dimethoxytrityl chloride (1.345 g, 3.96 mmol) was added, and the mixture was stirred at room temperature for 2 h. The reaction was quenched with MeOH (5 mL), EtOAc (150 mL) was added, and the mixture was washed successively with half saturated aqueous $NaHCO_3$ and H_2O. The aqueous layer was re-extracted with CH_2Cl_2 and EtOAc, the combined organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with acetonitrile (2 × 30 mL). The residue was purified by silica gel column chromatography (7.3 × 5.5 cm) with a gradient of 0.5:2.0−5.0:97.5−94.5 pyridine/MeOH/CH_2Cl_2 (v/v/v) as eluent to give **16** (1297 mg, 87.0%) as a white foam after coevaporation with acetonitrile (3 × 25 mL). R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.56; FAB-MS

m/z 574.1 $[M]^+$; ^1H NMR ($CDCl_3$) δ 10.00 (1H, bs, NH), 7.41 (2H, d, *J* 7.3 Hz, DMT), 7.31−7.16 (7H, m, DMT), 7.12 (1H, d, *J* 0.9 Hz, H6), 6.79 (4H, d, *J* 8.8 Hz, DMT), 4.07−3.98 (3H, m, H1′, H2′, H3′), 3.91−3.78 (3H, m, H1′, H5′), 3.74 (6H, s, OCH_3), 3.44 (1H, bs, OH), 3.43 (1H, d, *J* 9.7 Hz, H5″), 3.33 (1H, d, *J* 9.5 Hz, H5″), 1.96 (1H, d, *J* 5.5 Hz, 3′OH), 1.83 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) 164.60 (C4), 158.44 (DMT), 151.59 (C2), 144.41 (DMT), 142.00 (C6), 135.47, 129.96, 127.96, 127.81, 126.82, 113.14 (DMT), 110.06 (C5), 86.39 (CAr_2Ph), 84.71 (C2′), 81.90 (C4′), 79.17 (C3′), 75.52 (C5′), 63.61 (C5″), 55.11 (OCH_3), 49.87 (C1′), 12.15 (CH_3).

(2*S*,3*S*,4*R*)-3-(*tert*-Butyldimethylsilyloxy)-4-(4,4′-dimethoxytrityl)oxymethyl-4-hydroxy-2-((thymin-1-yl)methyl)tetrahydrofuran (17). Compound **16** (1266 mg, 1.84 mmol) was dissolved in anhydrous CH_2Cl_2 (50 mL) under a N_2 atmosphere, imidazole (604 mg, 8.87 mmol) and TBDMSCl (909 mg, 5.35 mmol) were added, and the mixture was stirred at room temperature. After 24 h, further TBDMSCl (380 mg) was added, and after additional 48 h, the reaction mixture was diluted with EtOAc (200 mL) and washed successively with saturated aqueous $NaHCO_3$ and H_2O. The organic phase was evaporated to dryness under reduced pressure and coevaporated with acetonitrile (2 × 10 mL), and the residue was purified by silica gel column chromatography (7.5 × 5.5 cm) using 0.5:20:79.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) as the eluent to give **17** (1042 mg, 68.6%) as a white foam after coevaporation with acetonitrile (2 × 15 mL). R_f (1:1 EtOAc/CH_2Cl_2 (v/v)) 0.56; FAB-MS *m/z* 689 $[M + H]^+$; ^1H NMR ($CDCl_3$) δ 8.6 (1H, bs, NH), 7.38−7.21 (9H, m, DMT), 7.14 (1H, d, *J* 1.3 Hz, H6), 6.85−6.81 (4H, m, DMT), 4.17 (1H, dd, *J* 3.1, 13.9 Hz, H1′), 4.01 (1H, d, *J* 9.5 Hz, H5′), 3.95 (1H, dt, *J* 2.7, 8.4 Hz, H2′), 3.85 (1H, d, *J* 9.5 Hz, H5′), 3.85 (1H, d, *J* 2.4 Hz, H3′), 3.79 (6H, s, OCH_3), 3.70 (1H, d, *J* 8.4, 13.9 Hz, H1′), 3.36 (1H, d, *J* 9.3 Hz, H5″), 3.30 (1H, d, *J* 9.5 Hz, H5″), 2.86 (1H, bs, OH), 1.91 (3H, d, *J* 1.1 Hz, CH_3), 0.71 (9H, s, $C(CH_3)_3$), 0.07, 0.02 (6H, 2s, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 164.04 (C4), 158.48 (DMT), 150.68 (C2), 144.27 (DMT), 141.80 (C6), 135.39, 135.35, 129.87, 127.92, 127.77, 126.86, 113.10 (DMT), 109.60 (C5), 86.41 (CAr_2Ph), 85.40 (C2′), 82.01 (C4′), 79.53 (C3′), 76.48 (C5′), 64.19 (C5″), 55.09 (OCH_3), 50.24 (C1′), 25.42 ($C(CH_3)_3$, 17.54 ($C(CH_3)_3$), 12.18 (CH_3), −4.85, −5.07 ($Si(CH_3)_2$).

(2*S*,3*S*,4*R*)-4-Acetoxy-3-(*tert*-butyldimethylsilyloxy)-4-(4,4′-dimethoxytrityl)oxymethyl-2-((thymin-1-yl)methyl)-tetrahydrofuran (18). Compound **17** (1.006 g, 1.46 mmol) was dissolved in anhydrous pyridine (20 mL) under a N_2 atmosphere. DMAP (1.428 mg, 11.7 mmol) and Ac_2O (1.0 mL, 10.6 mmol) were added, and the solution was stirred at room temperature for 18 h. H_2O (60 mL) was added, and the mixture was extracted with EtOAc. The combined organic phase was washed with saturated aqueous $NaHCO_3$ and H_2O and evaporated to dryness under reduced pressure, and the residue was coevaporated with acetonitrile (2 × 10 mL). The residue was subjected to silica gel column chromatography (15.5 × 3.3 cm) with 0.5:15−20:84.5−79.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) as the gradient eluent to give **18** (650 mg, 60.9%) as a yellowish foam after coevaporation with acetonitrile (3 × 15 mL). R_f (1:1 EtOAc/CH_2Cl_2 (v/v)) 0.73; ^1H ($CDCl_3$) δ 8.40 (1H, bs, NH), 7.38−7.35 (2H, m, DMT), 7.28−7.20 (7H, m, DMT), 7.16 (1H, d, *J* 1.3 Hz, H6), 6.81−6.78 (4H, m, DMT), 4.36 (1H, d, *J* 10.4 Hz, H5′), 4.30 (1H, d, *J* 4.6 Hz, H3′), 4.13 (1H, dd, *J* 2.9, 14.1 Hz, H1′), 3.92 (1H, d, *J* 10.4 Hz, H5′), 3.89 (1H, d, *J* 10.4 Hz, H5″), 3.83 (1H, m, H2′), 3.79 (6H, d, *J* 0.4 Hz, OCH_3), 3.57 (1H, d, *J* 7.8, 14.2 Hz, H1′), 3.24 (1H, d, *J* 10.4 Hz, H5″), 2.07 (3H, s, $COCH_3$), 1.92 (3H, d, *J* 1.3 Hz, CH_3), 0.73 (9H, s, $C(CH_3)_3$), 0.03, −0.09 (6H, 2s, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 170.06 ($COCH_3$), 163.78 (C4), 158.40 (DMT), 150.55 (C2), 144.61 (DMT), 141.38 (C6), 135.85, 135.55, 129.85, 129.81, 127.90, 127.66, 126.72, 112.99 (DMT), 109.90 (C5), 90.25 (C4′), 85.90 (CAr_2Ph), 84.88 (C2′), 77.53 (C3′), 74.86 (C5′), 61.62 (C5″), 55.06 (OCH_3), 49.17 (C1′), 25.47 ($C(CH_3)_3$, 21.80 ($COCH_3$) 17.61 ($C(CH_3)_3$), 12.20 (CH_3), −5.00, −5.27 ($Si(CH_3)_2$). Anal. Calcd for ($C_{40}H_{50}N_2O_9Si$) C, 65.7; H, 6.9; N, 3.8. Found: C, 65.8; H, 7.0; N, 4.1.

(2*S*,3*S*,4*R*)-4-Acetoxy-3-hydroxy-4-(4,4′-dimethoxytrityl)-oxymethyl-2-((thymin-1-yl)methyl)tetrahydrofuran (19). Compound **18** (623 mg, 0.85 mmol) was dissolved in anhydrous THF (20 mL), tetrabutylammonium fluoride in THF (5 mL, 1 M) was slowly added, and the mixture was stirred for 10 min. After evaporation to dryness under reduced pressure, the residue was purified by silica gel column chromatography (13.5 × 3.3 cm) eluting with a gradient of 0.5:25−35:74.5−64.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) to give **19** (533 mg) as a yellowish foam after coevaporation with acetonitrile (3 × 15 mL). R_f (1:1 EtOAc/CH_2Cl_2 (v/v)) 0.37; FAB-MS *m/z* 616 $[M]^+$; ^1H NMR ($CDCl_3$) δ 8.61 (1H, bs, NH), 7.38−7.36 (2H, m, DMT), 7.29−7.21 (7H, m, DMT), 7.09 (1H, d, *J* 1.1 Hz, H6), 6.84−6.80 (4H, m, DMT), 4.20 (1H, d, *J* 10.8 Hz, H5′), 4.11 (1H, d, *J* 11.4 Hz, H1′), 4.06 (1H, d, *J* 10.8 Hz, H5′), 4.01 (1H, m, H2′), 3.82−3.75 (2H, m, H1′, H3′), 3.79 (6H, s, OCH_3), 3.64 (1H, d, *J* 10.6 Hz, H5″), 3.49 (1H, d, *J* 10.8 Hz, H5″), 3.34 (1H, d, *J* 2.9 Hz, OH), 2.09 (3H, s, $COCH_3$), 1.90 (3H, d, *J* 1.1 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 171.64 ($COCH_3$), 163.86 (C4), 158.51 (DMT), 150.80 (C2), 144.25 (DMT), 141.04 (C6), 135.24, 135.17, 129.85, 127.84, 126.88, 113.12 (DMT), 110.18 (C5), 89.65 (C4′), 86.22 (CAr_2Ph), 81.16 (C3′), 79.29 (C2′), 74.05 (C5′), 61.26 (C5″), 55.10 (OCH_3), 48.87 (C1′), 21.54 ($COCH_3$), 12.20 (CH_3). This crude product contained impurities which could be assigned as Bu_4N^+ and *n*-hexane: ^1H NMR ($CDCl_3$) 3.05 (m), 1.75 (m), 1.40 (sextet), 0.99 (t, *J* 7.4 Hz) (Bu_4N^+), 1.26 (m), 0.88 (t, *J* 6.9 Hz) (*n*-hexane); ^{13}C NMR ($CDCl_3$) 32, 23.14 (*n*-hexane), 53.5, 25, 20, 13.5 (Bu_4N^+). The crude product was used in the next step without further purification.

(2*S*,3*S*,4*R*)-4-Acetoxy-3-(2-cyanoethoxy(diisopropylamino)phosphinoxy)-4-(4,4′-dimethoxytrityl)oxymethyl-2-((thymin-1-yl)methyl)tetrahydrofuran (20). Nucleoside **19** (310 mg, 0.50 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (10 mL) and $(i\text{-}Pr)_2NEt$ (2.5 mL) under a N_2 atmosphere. 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.6 mL, 2.7 mmol) was added, and the mixture was stirred at room temperature for 4 h. The reaction was quenched by the addition of H_2O (5 mL), the mixture was diluted with EtOAc (150 mL), and washing was performed using successively saturated $NaHCO_3$ and H_2O. The organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with acetonitrile (2 × 20 mL). The residue was purified by silica gel column chromatography (17.5 × 3.3 cm) using 0.5:24.5:75 pyridine/EtOAc/CH_2Cl_2 (v/v/v) as the eluent. Impure fractions (all fractions were analyzed by ^{31}P NMR) were purified once more on a silica gel column (14 × 3.3 cm) using a gradient of 0.5:15−25:74.5−64.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) as eluent. Fractions containing pure product were combined and evaporated to give amidite **20** (277 mg, 67.4% from **18**) as a colorless foam after coevaporation with acetonitrile (4 × 10 mL). R_f (1:1 EtOAc/CH_2Cl_2 (v/v)) 0.70; FAB-MS *m/z* 817 $[M + H]^+$; ^{31}P NMR (DMSO-d_6) δ 151.84, 151.74.

(2*S*,3*S*,4*S*)-3,4-Dihydroxy-4-hydroxymethyl-2-((*p*-toluenesulfonyloxy)methyl)tetrahydrofuran (21). Compound **7** (2.992 g, 6.00 mmol) was dissolved in anhydrous EtOH (20 mL), 20% $Pd(OH)_2/C$ (0.1 g) was added, and the mixture was degassed with H_2 under reduced pressure and stirred under a H_2 atmosphere. After 31 h, the mixture was evaporated to dryness under reduced pressure, and the residue was subjected to silica gel column chromatography (7.8 × 4.8 cm) using 5% MeOH in CH_2Cl_2 (v/v) as eluent to give **21** (1.642 g, 86.0%) as a white solid material. R_f (10% MeOH in CH_2Cl_2) 0.39; FAB-MS *m/z* 319 $[M + H]^+$; ^1H NMR (CD_3OD) δ 7.79 (2H, d, *J* 8.2 Hz, Ar), 7.43 (2H, d, *J* 8.0 Hz, Ar), 4.15 (2H, m, H1), 3.89 (1H, m, H2), 3.82 (1H, m, H3), 3.82 (1H, d, *J* 9.2 Hz, H5), 3.71 (1H, d, *J* 11.5 Hz, H5′), 3.70 (1H, d, *J* 9.2 Hz, H5), 3.62 (1H, d, *J* 11.5 Hz, H5′), 2.45 (3H, s, $ArCH_3$); ^{13}C NMR (CD_3OD) δ 144.60, 132.35, 129.12, 127.12 (Ar), 83.75 (C2), 80.96 (C4), 77.71 (C1), 74.16 (C5), 69.74 (C1), 61.99 (C5′), 19.67 ($ArCH_3$). Anal. Calcd for ($C_{13}H_{18}O_7S$) C, 48.7; H, 5.7. Found: C, 49.0; H, 5.7.

(2*S*,3*S*,4*R*)-3,4-Dihydroxy-4-hydroxymethyl-3-*O*,*O*-(hydroxymethyl)-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-((*p*-toluenesulfonyloxy)methyl)tetrahydrofuran (22). Compound **21** (2.401 g, 7.54 mmol) was dissolved in anhydrous pyridine (25 mL) under a N_2 atmosphere, and 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (2.8 mL, 9.0 mmol) was added.

The mixture was stirred at room temperature for 35 h whereupon H_2O (150 mL) was added. The suspension was extracted with EtOAc, and the combined organic phase was washed successively with saturated aqueous $NaHCO_3$ and H_2O. The organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with anhydrous toluene (3 × 15 mL). The residue was subjected to silica gel column chromatography (13.8 × 5.5 cm) eluting with a gradient of 10−15% EtOAc in petroleum ether (v/v) to give **22** (3.015 g, 71.3%) as a white solid material. R_f (3:7 EtOAc/petroleum ether (v/v)) 0.50; FAB-MS *m*/*z* 561 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) 7.78 (2H, d, *J* 8.4 Hz, Ar), 7.32 (2H, d, *J* 8.1 Hz, Ar), 4.25 (1H, m, H1), 4.12 (2H, m, H2, H1), 4.03 (1H, d, *J* 11.0 Hz, H5′), 3.98 (1H, s, H3), 3.74 (1H, d, *J* 9.3 Hz, H5), 3.65 (1H, d, *J* 9.3 Hz, H5), 3.54 (1H, d, *J* 11.2 Hz, H5′), 2.8 (1H, br s, OH), 2.44 (3H, s, $ArCH_3$), 1.09−0.96 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$) 144.64, 132.83, 129.65, 127.86 (Ar), 85.03 (C2), 83.61 (C4), 77.56 (C3), 72.94 (C5), 69.17 (C1), 60.68 (C5′), 21.53 ($ArCH_3$), 17.14, 17.10, 17.06, 13.28, 12.51, 12.39, 12.21 (TIPDS). Anal. Calcd for ($C_{25}H_{44}O_8SSi_2$) C, 53.5; H, 7.9. Found: C, 53.5; H, 7.8.

(2*S*,3*S*,4*R*)-3-Hydroxy-4-hydroxymethyl-4-*O*-methoxalyloxy-3-*O*,*O*-(hydroxymethyl)-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-((*p*-toluenesulfonyl)methyl)tetrahydrofuran (23). Compound **22** (559 mg, 1.00 mmol) was dissolved in anhydrous CH_2Cl_2 (10 mL) under a N_2 atmosphere at 0 °C. DMAP (1.047 g, 8.57 mmol) followed by methoxalyl chloride (0.30 mL, 3.26 mmol) were added, and the mixture was stirred at room temperature for 22 h. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography (6.5 × 5.8 cm) using CH_2Cl_2 as the eluent to give **23** (480 mg, 74.4%) as a colorless oil. R_f (0.5% CH_3OH in CH_2Cl_2 (v/v)) 0.70; FAB-MS *m*/*z* 647 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.80 (2H, d, *J* 8.2 Hz, Ar), 7.33 (2H, d, *J* 8.2 Hz, Ar), 4.54 (1H, d, *J* 12.3 Hz, H5′), 4.40 (1H, d, *J* 11.0 Hz, H5), 4.27−4.22 (2H, m, H3, H1), 4.13−4.08 (2H, m, H1, H2), 4.01 (1H, d, *J* 12.3 Hz, H5′), 3.89 (3H, s, OCH_3), 3.67 (1H, d, *J* 11.0 Hz, H5), 2.45 (3H, s, $ArCH_3$), 1.07−0.91 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$) δ 157.34, 155.29 (C═O), 144.71, 132.82, 129.64, 127.83 (Ar), 94.67 (C4), 84.68 (C2), 75.82 (C3), 69.21 (C5), 68.53 (C1), 56.00 (C5′), 53.45 (OCH_3), 21.52 ($ArCH_3$), 17.09, 17.05, 17.00, 16.79, 13.22, 12.35, 12.34, 12.21 (TIPDS).

(2*S*,3*S*,4*S*)-3-Hydroxy-4-hydroxymethyl-3-*O*,*O*-(hydroxymethyl)-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-((*p*-toluenesulfonyloxy)methyl)tetrahydrofuran (24) and (2*S*,3*S*,4*R*)-3-Hydroxy-4-hydroxymethyl-3-*O*,*O*-(hydroxymethyl)-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-((*p*-toluenesulfonyloxy)methyl)tetrahydrofuran (24a). Compound **23** (406 mg, 0.628 mmol) was dissolved in anhydrous toluene (20 mL), the volume of the mixture was reduced to 75% under reduced pressure, and the resulting mixture was heated to 110 °C under a N_2 atmosphere. Bu_3SnH (0.5 mL, 1.86 mmol) and AIBN (37 mg, 0.22 mmol) were added, and the mixture was heated under reflux for 1 h. After being cooled to room temperature, the mixture was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography (11.0 × 3.3 cm) using CH_2Cl_2 as the eluent to give **24** (197 mg, 57.6%) and **24a** (20 mg, 6%) as colorless oils. In addition, a 2:1 mixture of **24** and **24a** (68 mg, 20%) was obtained. R_f (3:7 EtOAc/petroleum ether (v/v)) 0.55. Data for **24**: FAB-MS *m*/*z* 545 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.77 (2H, d, *J* 8.2 Hz, Ar), 7.33 (2H, d, *J* 8.6 Hz, Ar), 4.33 (1H, d, *J* 4.6 Hz, H3), 4.07 (1H, dd, *J* 4.6, 6.0 Hz, H2), 4.01 (1H, dd, *J* 4.3, 10.3 Hz, H1), 3.91 (1H, dd, *J* 6.2, 10.3 Hz, H1), 3.84−3.78 (2H, m, H5, H5′), 3.69 (1H, dd, *J* 4.2, 10.8 Hz, H5′), 3.44 (1H, dd, *J* 7.8, 11.9 Hz, H5), 2.45 (3H, s, $ArCH_3$), 2.40 (1H, m, H4), 1.08−0.93 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$) δ 144.76, 132.62, 129.68, 127.79 (Ar), 85.10 (C2), 72.82 (C3), 68.90 (C1), 67.99 (C5), 57.19 (C5′), 47.16 (C4), 21.51 ($ArCH_3$), 17.22, 17.15, 17.04, 16.99, 13.25, 12.52, 12.46, 12.34 (TIPDS). **24a**: FAB-MS *m*/*z* 545 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 7.79 (2H, d, *J* 8.4 Hz, Ar), 7.33 (2H, d, *J* 8.1 Hz, Ar), 4.30 (1H, dd, *J* 2.4, 10.6 Hz, H1), 4.18 (1H, dd, *J* 7.1 Hz, H3), 4.09 (1H, dd, *J* 4.8, 10.6 Hz, H1), 3.95 (1H, d, *J* 8.6 Hz, H5), 3.90 (1H, dd, *J* 4.3, 12.0 Hz, H5′), 3.82 (1H, ddd, *J* 2.4, 4.8, 9.9 Hz, H2), 3.66 (1H, dd, *J* 7.8, 11.8 Hz, H5′), 3.48 (1H, t, *J* 8.5 Hz, H5), 2.44 (3H, s, $ArCH_3$), 2.43−2.37 (1H, m, H4), 1.08−0.87 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$) δ 144.58, 132.86, 129.59, 127.86 (Ar), 81.89 (C2), 73.68 (C3), 68.85 (C1), 68.47 (C5), 61.80 (C5′), 49.55 (C4), 21.51 ($ArCH_3$), 17.40−16.91, 13.37−12.35 (TIPDS).

(2*S*,3*S*,4*S*)-3-Hydroxy-4-hydroxymethyl-3-*O*,*O*-(hydroxymethyl)-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-((thymin-1-yl)methyl)tetrahydrofuran (25). Thymine (454 mg, 3.60 mmol) was dissolved in anhydrous DMF (20 mL) at 120 °C under a N_2 atmosphere, NaH (143 mg, 60% w/w, 3.58 mmol) was added, and the suspension was stirred for 5 min. Compound **24** (978 mg, 1.19 mmol) dissolved in anhydrous DMF (40 mL) was slowly added, and the mixture was stirred at 120 °C for 2.5 h. After being cooled to room temperature, H_2O (100 mL) was added, and the mixture was extracted with EtOAc. The combined organic phase was washed successively with saturated aqueous $NaHCO_3$ and H_2O, evaporated to dryness under reduced pressure, and the residue was coevaporated with *n*-hexane (10 mL) and toluene (10 mL). The residue was purified by silica gel column chromatography (14.5 × 3.3 cm) eluting with a gradient of 30−50% EtOAc in petroleum ether (v/v) to give **25** (548 mg, 61.2%) as a white solid material after coevaporation with CH_2Cl_2 (3 × 15 mL). R_f (1:1 EtOAc/petroleum ether (v/v)) 0.31; FAB-MS *m*/*z* 499 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.94 (1H, bs, NH), 7.10 (1H, d, *J* 0.7 Hz, H6), 4.39 (1H, d, *J* 4.4 Hz, H3′), 4.19 (1H, dd, *J* 4.3, 5.5 Hz, H2′), 3.92−3.82 (3H, m, H1′, H5′, H5″), 3.76−3.70 (2H, m, H5″, H1′), 3.51 (1H, dd, *J* 8.1, 11.9 Hz, H5′), 2.30 (1H, m, H4′), 1.91 (3H, s, CH_3), 1.07−0.87 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$) δ 163.65 (C4), 150.57 (C2), 140.97 (C6), 110.21 (C5), 86.38 (C2′), 72.93 (C3′), 67.88 (C5′), 57.13 (C5″), 49.72 (C1′), 47.90 (C4′), 17.25, 17.21, 17.15, 16.98, 16.92 (TIPDS), 13.31 (CH_3), 12.65, 12.40, 12.34, 12.19 (TIPDS).

(2*S*,3*S*,4*R*)-3-Hydroxy-4-hydroxymethyl-3-*O*,(*O*-hydroxymethyl)-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-((thymin-1-yl)methyl)tetrahydrofuran (25a). In analytical scale using conditions similar to those described above for the preparation of **25** but reacting with a mixture of **24** and **24a**, **25a** was obtained in a pure form after column chromatographic purification eluting with a gradient of 20−30% EtOAc/petroleum ether (v/v). FAB-MS *m*/*z* 499 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 8.64 (1H, bs, NH), 7.16 (1H, d, *J* 0.9 Hz, H6), 4.34 (1H, dd, *J* 2.2, 14.1 Hz, H1′), 4.05−3.93 (3H, m, H3′, H5′, H5″), 3.88 (1H, ddd, *J* 2.2, 7.8, 8.5 Hz, H2′), 3.65 (1H, dd, *J* 8.1, 11.8 Hz, H5″), 3.55 (1H, dd, *J* 7.0, 9.2 Hz, H5′), 3.50 (1H, dd, *J* 8.6, 14.1 Hz, H1′), 2.40 (1H, m, H4′), 1.90 (3H, d, *J* 0.7 Hz, CH_3), 1.09−0.94 (28H, m, TIPDS); ^{13}C NMR ($CDCl_3$) 163.94 (C4), 150.59 (C2), 141.03 (C6), 109.96 (C5), 82.69 (C2′), 75.77 (C3′), 68.34 (C5′), 62.26 (C5″), 49.53 (C1′, C4′), 17.43−16.94, 13.57, 13.31, 12.90, 12.51, 12.22 (TIPDS, CH_3). Anal. Calcd for ($C_{23}H_{42}N_2O_6Si_2$) C, 55.4; H, 8.5; N, 5.6. Found: C, 55.4; H, 8.5; N, 5.5.

(2*S*,3*S*,4*R*)-3-Hydroxy-4-hydroxymethyl-2-((thymin-1-yl)methyl)tetrahydrofuran (26). Compound **25** (502 mg, 1.01 mmol) was dissolved in anhydrous THF (20 mL) under a N_2 atmosphere at room temperature. A solution of tertrabutylammonium fluoride in THF (1.0 M, 5 mL, 5.0 mmol) was slowly added, and the mixture was stirred for 10 min. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography (13.5 × 3.3 cm) using 10% MeOH/CH_2Cl_2 (v/v) as the eluent, affording **26** (397 mg) as a white foam after coevaporation with *n*-hexane (3 × 10 mL). R_f (20% MeOH in CH_2Cl_2 (v/v)) 0.51; FAB-MS *m*/*z* 257 $[M + H]^+$; ^{1}H NMR (CD_3OD) 7.45 (1H, q, *J* 0.9 Hz, H6), 4.21 (1H, dd, *J* 2.9, 6.0 Hz, H3′), 4.09 (1H, dd, *J* 7.7, 8.4 Hz, H5′), 4.05 (1H, ddd, *J* 3.3, 4.2, 10.6 Hz, H2′), 4.00 (1H, dd, *J* 4.2, 13.9 Hz, H1′), 3.88 (1H, dd, *J* 6.3, 10.9 Hz, H5″), 3.78−3.69 (2H, m, H5′, H1′), 3.67 (1H, dd, *J* 7.4, 10.9 Hz, H5″), 2.43 (1H, m, H4′), 1.90 (3H, d, *J* 1.1 Hz, CH_3); ^{13}C NMR (CD_3OD) 164.88 (C4), 151.08 (C2), 142.01 (C6), 108.67 (C5), 83.73 (C2′), 72.85 (C3′), 69.16 (C5′), 58.22 (C5″), 49.01 (C1′), 45.04 (C4′), 10.30 (CH_3). This product contained impurities which could be assigned as Bu_4N^+ (^{1}H NMR (CD_3OD) 3.30−3.26 (2H, m), 1.70 (2H, quintet), 1.46 (2H,

sextet, *J* 7.3 Hz), 1.07 (3H, t, *J* 7.3 Hz); ^{13}C NMR (CD_3OD) 57.63−57.57, 22.89, 18.81, 12.03; FAB-MS *m*/*z* 242 (Bu_4N^+)). This crude product was used in the next step without further purification.

(2*S*,3*S*,4*S*)-4-(4,4′-Dimethoxytrityl)oxymethyl-3-hydroxy-2-((thymin-1-yl)methyl)tetrahydrofuran (27). Crude product **26** (371 mg) in anhydrous pyridine (20 mL) was concentrated under reduced pressure to ca. three-quarters volume, and the resulting mixture was cooled to 0 °C under a N_2 atmosphere. 4,4′-Dimethoxytrityl chloride (970 mg, 2.85 mmol) was added, and after being stirred at room temperature for 2 h, MeOH (3 mL) was added. The mixture was evaporated to dryness under reduced pressure, coevaporated with acetonitrile (2 × 20 mL), and the residue was purified by silica gel column chromatography (17.0 × 3.3 cm) using 0.5:25−60:74.5−39.5 pyridine/EtOAc/CH_2Cl_2 (v/v/v) as the gradient eluent to give **27** (333 mg, 63.4% from **25**) as a white foam after coevaporation with acetonitrile (2 × 10 mL) and *n*-hexane (2 × 10 mL). R_f (10% MeOH in CH_2Cl_2 (v/v)) 0.47; FAB-MS *m*/*z* 558.1 $[M]^+$; ^{1}H NMR ($CDCl_3$) δ 9.27 (1H, bs, NH), 7.41−7.39 (2H, m, DMT), 7.31−7.18 (7H, m, DMT), 7.09 (1H, d, *J* 0.9 Hz, H6), 6.84−6.81 (4H, m, DMT), 4.16 (1H, m, H3′), 4.05−3.92 (3H, m, H5′, H1′, H2′), 3.78 (6H, s, OCH_3), 3.74−3.67 (2H, m, H5′, H1′), 3.32 (2H, d, *J* 6.8 Hz, H5″), 3.04 (1H, d, *J* 4.4 Hz, OH), 2.47 (1H, m, H4′), 1.89 (3H, d, *J* 0.7 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.16 (C4), 158.35 (DMT), 151.27 (C2), 144.46 (DMT), 141.20 (C6), 135.65, 135.54, 129.75, 127.80, 127.74, 126.70, 113.06 (DMT), 110.23 (C5), 86.40 (*C*PhAr$_2$), 83.67 (C2′), 73.84 (C3′), 69.78 (C5′), 60.37 (C5″), 55.06 (OCH_3), 49.24 (C1′), 43.17 (C4′), 12.17 (CH_3).

(2*S*,3*S*,4*S*)-3-(2-Cyanoethoxy(diisopropylamino)-phosphinoxy)-4-((4,4′-dimethoxytrityl)oxymethyl)-2-((thymin-1-yl)methyl)tetrahydrofuran (28). Compound **27** (286 mg, 0.512 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (10 mL) and *N*,*N*-diisopropylethylamine (2.5 mL) under a N_2 atmosphere at 0 °C. 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (0.6 mL, 2.69 mmol) was added, and the mixture was stirred at room temperature for 4 h. H_2O (5 mL) and EtOAc (150 mL) were added, and the solution was washed successively with saturated aqueous $NaHCO_3$ and H_2O. The combined organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with acetonitrile (2 × 20 mL). The residue was purified by silica gel column chromatography (17.0 × 3.3 cm) using 0.5:24.5:75 pyridine/EtOAc/CH_2Cl_2 (v/v/v) as the eluent, yielding amidite **29** (263 mg, 67.8%) as a white foam after coevaporation with acetonitrile (3 × 20 mL). R_f (1:1 EtOAc/CH_2Cl_2 (v/v)) 0.52; FAB-MS *m*/*z* 759 $[M + H]^+$; ^{31}P NMR (DMSO-d_6) 150.62, 148.63.

Oligonucleotide Synthesis. All oligomers were synthesized on a DNA synthesizer using the phosphoramidite approach.[31] The stepwise coupling yields were determined spectrophotometrically at 498 nm (quantifying the released 4,4′-dimethoxytrityl group). The modified amidites **11**, **20**, and **28** were "hand-coupled" (premixing a ∼0.05 M solution of amidite in anhydrous acetonitrile (0.2 mL, 10 μmol) and a ∼0.5 M solution of tetrazole in anhydrous acetonitrile (0.3 mL, 150 μmol) in a syringe and via an adaptor slowly flushing this mixture through a synthesis column for the coupling times indicated below). The results for the modified amidites were as follows: >99% stepwise yield (6 min coupling time) for amidite **11**, >98% stepwise yield (15 min coupling time) for amidite **20**, and 95−99% stepwise yield (20 min coupling time) for amidite **28**. The stepwise coupling yields for the unmodified amidites and the amidites leading to incorporation of the $\mathbf{T^L}$ and **X** monomers were >99% (standard conditions were used except for 6 min coupling time for the amidites leading to incorporation of the $\mathbf{T^L}$ and **X** monomers). However, the coupling with an unmodified RNA amidite (2′-*O*-TBDMS-protected RNA amidites were used) following an incorporation of amidite **20** and amidite **28** proceeded in only 95−99% and 92−98% stepwise yield, respectively, even when using the hand-coupling conditions described above (up to 20 min coupling time). Cleavage from the solid support and removal of protecting groups was in general accomplished using concentrated aqueous ammonia (55 °C for 16 h). For all sequences containing monomer $\mathbf{Z^L}$ or RNA monomers, methylamine (40% (w/w); 10 min, 55 °C) was used instead. 13-Mers were purified by ethanol precipitation and 9-mers by desalting (using a Pharmacia NAP column). All sequences containing RNA monomers were desilylated and subsequently purified by 1-butanol precipitation as described earlier.[36] Capillary gel electrophoresis of all synthesized sequences showed >90% purity. The compositions were verified by MALDI-MS analysis (*m*/*z* $[M-H]^-$: found mass; calcd mass): 5′-*d*(GTGA$\mathbf{X^L}$ATGC); 2658; 2656.7; 5′-*d*(GTGA**X**ATGC); 2631; 2628.7; 5′-*r*(GUGA$\mathbf{X^L}$AUGC); 2760; 2756.3; 5′-*d*(CAGTGA**X**ATGCGA); 3875; 3873.5; 5′-*d*(CAG$\mathbf{X^L}$GA$\mathbf{X^L}$A$\mathbf{X^L}$GCGA); 3710; 3709.1; 5′-*r*(CAG$\mathbf{X^L}$GA$\mathbf{X^L}$A$\mathbf{X^L}$GCGA); 3870; 3869.1; 5′-*d*(CAGTGA$\mathbf{X^L}$ATGCGA); 3902; 3901.5; 5′-*r*(CAGUGA$\mathbf{X^L}$AUGCGA); 4069; 4065.1; 5′-*r*(CAGUGA**X**AUGCGA); 4038; 4037.1; 5′-*d*(GTGA$\mathbf{Z^L}$ATGC); 2783.6; 2783.7; 5′-*d*(GTGA$\mathbf{dZ^L}$ATGC); 26765.7; 2766.7; 5′-*d*(G$\mathbf{Z^L}$GA$\mathbf{Z^L}$A$\mathbf{Z^L}$GC); 2842.4; 2842.4; 5′-*d*(G$\mathbf{dZ^L}$GA$\mathbf{dZ^L}$A$\mathbf{dZ^L}$GC); 2794.7; 2794.5; 5′*d*(GCATATCAC); 2682.0; 2681.8; 5′-*r*(GUGA$\mathbf{Z^L}$AUGC); 2883.1; 2882.3; 5′-*r*(GUGA$\mathbf{dZ^L}$AUGC); 2866.9; 2866.3; 5′-*r*(G$\mathbf{Z^L}$GA$\mathbf{Z^L}$A$\mathbf{Z^L}$GC); 2939.1; 2938.4; 5′-*r*(G$\mathbf{dZ^L}$GA$\mathbf{dZ^L}$A$\mathbf{dZ^L}$GC); 2890.3; 2890.4; 5′-*d*(CAGTGA$\mathbf{Z^L}$ATGCGA); 4030.4; 4027.5; 5′-*d*(CAGTGA$\mathbf{dZ^L}$ATGCGA); 4013.2; 4011.5; 5′-*d*(CAG$\mathbf{Z^L}$GA$\mathbf{Z^L}$A$\mathbf{Z^L}$GCGA); 4087.9; 4087.2; 5′-*d*(CAG$\mathbf{dZ^L}$GA$\mathbf{dZ^L}$A$\mathbf{dZ^L}$GCGA); 4039.2; 4039.3; 5′-*d*($T_7\mathbf{Z^L}T_6$); 4222.1; 4225.6; 5′-*d*($T_7\mathbf{dZ^L}T_6$); 4206.5; 4209.6; 5′-*d*(TCGCATATCACTG); 3907.5; 3908.6; 5′-*r*(CAGUGA$\mathbf{Z^L}$AUGCGA); 4191.2; 4191.1; 5′-*r*(CAGUGA$\mathbf{dZ^L}$AUGCGA); 4176.3; 4175.1; 5′-*r*(CAG$\mathbf{Z^L}$GA$\mathbf{Z^L}$A$\mathbf{Z^L}$GCGA); 4247.2; 4247.2; 5′-*r*(CAG$\mathbf{dZ^L}$GA$\mathbf{dZ^L}$A$\mathbf{dZ^L}$GCGA); 4198.5; 4199.2.

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Supporting Information Available: Copies of ^{13}C NMR spectra for **1**, **3**, **6**−**10**, **12**−**19**, **21**−**27**, **24a**, and **25a** and copies of the ^{31}P NMR spectra for **11**, **20**, and **28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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