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3′-C-Branched LNA-Type Nucleosides Locked in an N-Type Furanose Ring Conformation: Synthesis, Incorporation into Oligodeoxynucleotides, and Hybridization Studies

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Three protected 3′-C-branched LNA-type phosphoramidite building blocks **17**, **27**, and **38**, containing furanose rings locked in an N-type conformation, were synthesized from a known 3-*C*-allyl allofuranose derivative using strategies relying on the introduction of the branching alkyl chain before condensation with the nucleobase. Synthesis of 3′-*C*-hydroxypropyl derivatives proved superior to synthesis of the 3′-*C*-hydroxyethyl derivatives, and the former was converted into the corresponding 3′-*C*-aminopropyl derivatives. Phosphoramidites **27** and **38** were subsequently applied on an automated DNA synthesizer leading to the introduction of three novel 3′-C-branched LNA-type monomers **X**, **Y**, and **Z** into oligodeoxynucleotides and studies of their effect on the hybridization properties. A duplex-stabilizing effect of introducing 3′-*C*-aminopropyl-LNA monomer **Y**, relative to 3′-*C*-hydroxypropyl-LNA monomer **X**, was observed, especially at low salt conditions. This indicates that the primary amino group of monomer **Y** is protonated under the hybridization conditions applied and that positioning of this positively charged group in the major groove has a significant duplex stabilizing effect. Monomer **Y** was by an on-column conjugation method further functionalized by a glycyl unit to give monomer **Z** that showed a less stabilizing effect than monomer **Y**.

Introduction

The antisense strategy involving targeting of single-stranded RNA has stimulated the synthesis and biophysical evaluation of a large number of modified oligonucleotides and oligodeoxynucleotides (ONs),[1−5] including LNA (locked nucleic acid; Figure 1, monomer **1**).[6−10] The conformation of the furanose moiety in an LNA monomer is locked in an N-type (C3′-*endo*) conformation ($P = 17°$),[8] the incorporation of one or more LNA monomers into an ON strongly increases the thermal stability of the duplex formed with single-stranded DNA or RNA complements,[6−9] and LNA has been shown to be very useful for antisense applications.[10] In an effort to further investigate the versatility of LNA monomers, three branched monomers have been synthesized to explore the opportunity of attaching at an internally positioned monomer in a

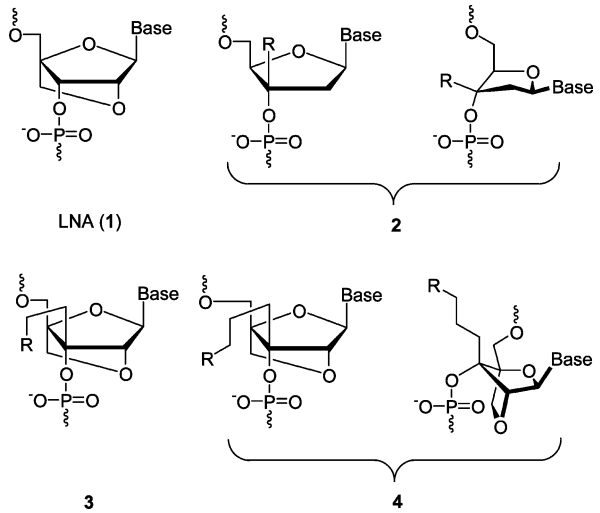

FIGURE 1. Constitution of LNA (**1**), 3′-C-branched-DNA (**2**), 3′-*C*-ethyl-LNA (**3**), and 3′-*C*-propyl-LNA (**4**) and a sketch of the *S*-type furanose conformation of monomer **2** and the N-type furanose ring conformation of monomers **3** and **4**.

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[†] University of Copenhagen.
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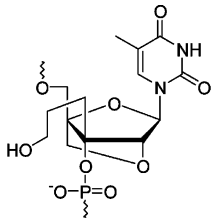
TABLE 1. ONs Studied and Thermal Denaturation Studies[a]

DNAs and LNAs studied

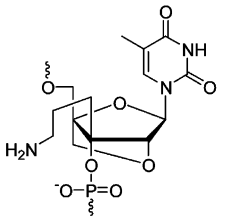
DNA-1	5'-d(GTGATATGC)
LNA-1	5'-d(G**X**GA**X**A**X**GC)
LNA-2	5'-d(G**Y**GA**Y**A**Y**GC)
LNA-3	5'-d(GTGA**Y**ATGC)
LNA-4	5'-d(GTGA**Z**ATGC)



X

T_m values in medium salt buffer ([Na^+] = 110 mM)

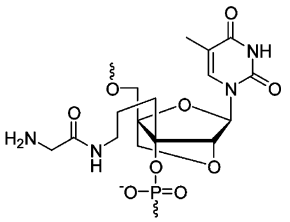
	Complementary DNA		Complementary RNA	
	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)
DNA-1	28	Ref.	25[b]/27[c]	Ref.
LNA-1	24	-1.3	27[b]	+0.7
LNA-2	32	+1.3	35[c]	+2.7
LNA-3	32	+4.0	27[c]	±0.0
LNA-4	27	-1.0	26[c]	-1.0



Y

T_m values in low salt buffer ([Na^+] = 10 mM)

	Complementary DNA		Complementary RNA	
	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)
DNA-1	11	Ref.	<5	Ref.
LNA-1	<5	<-2	12	>+2.3
LNA-2	21	>+3.3	24	>+6.3
LNA-3	15	+4.0	13	>+8.0
LNA-4	11	±0.0	10	>+5.0



Z

[a] 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) or in *low salt hybridization buffer* (10 mM sodium phosphate and 0.1 mM EDTA, pH 7.0) using 1.0 μM concentrations of the two strands assuming identical extinction coefficients of modified and unmodified nucleotides. ΔT_m values are calculated changes in T_m values per modification introduced relative to the reference **DNA-1**. [b,c] Measured in individual experimental series.

duplex, e.g., a third ON strand, a reporter molecule, or a positively charged group in an orientation facing the major groove of the duplex. Especially appealing is the option of attaching a positively charged group in order to mediate full or partial neutralization of an ON for increased duplex stability or improved cellular uptake. Several ONs containing amino-functionalized side chains attached to the furanose moiety have been studied, including 4′-C-,[11,12] 3′-C-,[12,13] and 2′-O-functionalized[14] derivatives. However, only moderately increased thermal stability of duplexes toward complementary DNA or RNA targets has at best been obtained.[11−14]

Introduction of a 3′-*C*-alkyl substituent (monomer **2**, Figure 1) on the β-face of a nucleotide monomer is well tolerated in a duplex structure as evaluated by the thermal stability of the duplexes between such modified ONs and a complementary sequence.[15−17] Therefore, it was appealing to study this structural feature in the context of an LNA monomer that in its parent unbranched form is known to very positively increase the thermal stability of duplexes.[6−9] We therefore decided to synthesize 3′-C-branched LNA monomers (**3** and **4**, Figure 1) with different 3′-*C*-alkyl branches. In a preliminary study, the usefulness of the branched LNA monomer **3** (R = oxygen linked to another ON strand) was demonstrated for the construction of a branched ON being able to form a bimolecular triple helical structure with a target strand.[18] Synthesis of the phosphoramidite building block for incorporation of the monomer applied in this study is described in this report for the first time. Furthermore, in an attempt at improving the accessibility of 3′-C-branched LNA monomers, we have synthesized phosphoramidite derivatives **27** and **38** containing propyl branches and studied the effect of the corresponding LNA-type monomers **X**, **Y**, and **Z** (see Table 1) on duplex stabilities when incorporated into ONs. A 3′-C-branched DNA monomer is known to adopt a C2′-*endo*-type fura-

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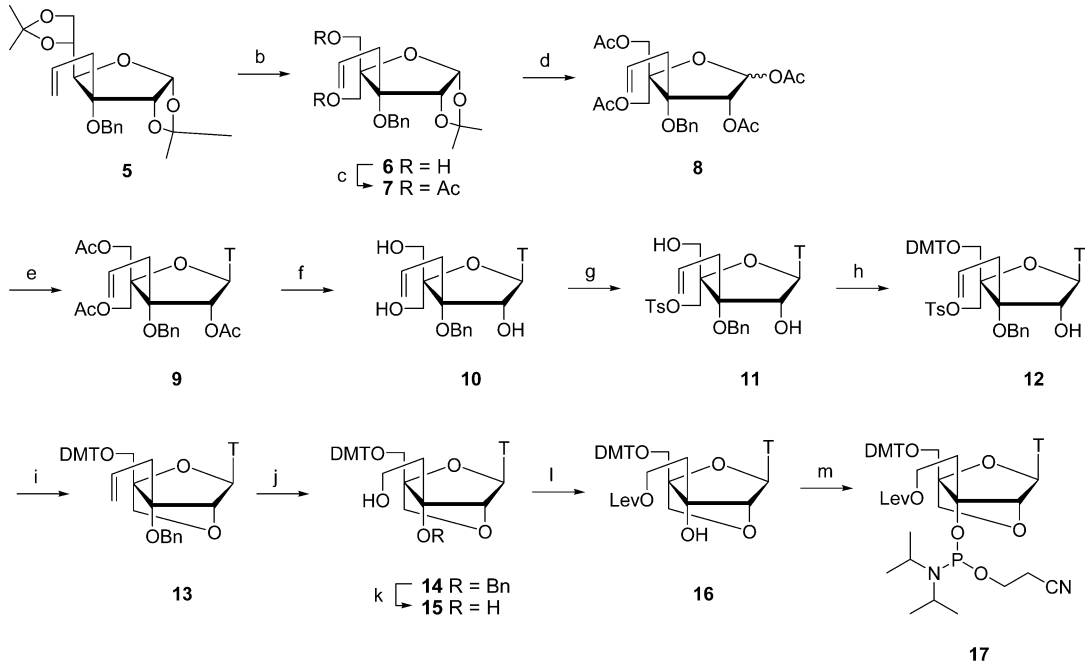
SCHEME 1[a]



[a] Key: (a) NaH, BnBr, DMF, 95% (synthesis of furanose **5** from 3-*C*-allyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose;[22] (b) (i) 80% aq AcOH or 60% aq AcOH, MeOH, (ii) $NaIO_4$, THF, H_2O, (iii) HCHO, NaOH, dioxane, H_2O, 59%; (c) Ac_2O, DMAP, pyridine, 85%; (d) (i) 80% aq AcOH, (ii) Ac_2O, DMAP, pyridine, 88%; (e) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, MeCN, 67%; (f) NaOMe, MeOH, 81%; (g) TsCl, pyridine, 70%; (h) DMTCl, pyridine, 74%; (i) NaH, DMF, 96%; (j) (i) OsO_4, $NaIO_4$, THF, H_2O, (ii) $NaBH_4$, THF/H_2O, 38%; (k) $HCOONH_4$, Pd/C, MeOH, 83%; (l) levulinic acid anhydride, pyridine, DMAP, 88%; (m) $(^iPr)_2NP(Cl)OCH_2CH_2CN$, $(^iPr)_2NEt$, CH_2Cl_2, 41%. T = Thymin-1-yl.

nose conformation[19] (Figure 1, monomer **2**), whereas 3′-C-branched LNA monomers will adopt a locked C3′-*endo*-type furanose conformation dictated by its bicyclic constitution. Therefore, this study is also aimed at studying the correlation between 3′-C-branching and furanose conformation.

Results and Discussion

Synthesis of 3′-*C*-Hydroxyethyl LNA-Type Nucleosides. For the introduction of the branch at C3′ of the nucleoside, a convergent synthesis strategy was chosen in order to obtain the desired configuration at C-3′. Thus, 3-*C*-allyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose was prepared in two steps from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose by oxidation and subsequent Grignard addition of allylMgBr to the ulose as previously described.[20−22] Benzylation of the tertiary hydroxyl group to give **5** was achieved in quantitative yield by reaction of 3-*C*-allyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose with sodium hydride and benzyl bromide in DMF. The subsequent selective removal of the 5,6-*O*-isopropylidene protective group using either 80% aqueous acetic acid at rt overnight or 60% aqueous acetic acid with a catalytic amount of methanol at 60 °C for 3 h[22] afforded the 5,6-diol. Oxidative cleavage using $NaIO_4$ in a mixture of THF and H_2O and subsequent aldol condensation between the resulting aldehyde and formaldehyde followed by reduction under in situ Cannizzaro reaction conditions afforded the diol **6** in 59% yield. The two primary hydroxyl groups were acetylated using acetic anhydride in pyridine with a catalytical amount of DMAP to give the diacetylated furanose derivative **7** in 85% yield. Acetolysis and subsequent acetylation afforded the tetra-*O*-acetyl sugar **8**, which was used as a glycosyl donor in a modified Vorbrüggen condensation reaction. Thus, the anomeric mixture **8** was stirred at 60 °C for 12 h with silylated thymine and TMS-triflate to give the nucleoside **9** (67% yield), obtained solely as the β-anomer due to achimeric assistance from the 2-*O*-acetyl group. The acylated nucleoside was deprotected using sodium methoxide in methanol to give nucleoside **10**. For activation of the 4′-*C*-hydroxymethyl moiety, a tosyl group was chosen as a leaving group. This could be introduced selectively by reacting **10** with tosyl chloride in analogy with the previously described selective reactions on this position.[23−25] Thus, nucleoside **10** was reacted with *p*-toluenesulfonyl chloride in pyridine which afforded the 4′-*C*-tosyloxymethyl nucleoside **11** in 70% yield (Scheme 1). The site of tosylation proved impossible to verify by NMR techniques and was therefore identified by an alternative approach taking advantage of the fact that the signals of the 1′-H, 2′-H, and 3′-H protons appear as singlets in the ^1H NMR spectra of LNA type nucleosides.[7] Thus, only

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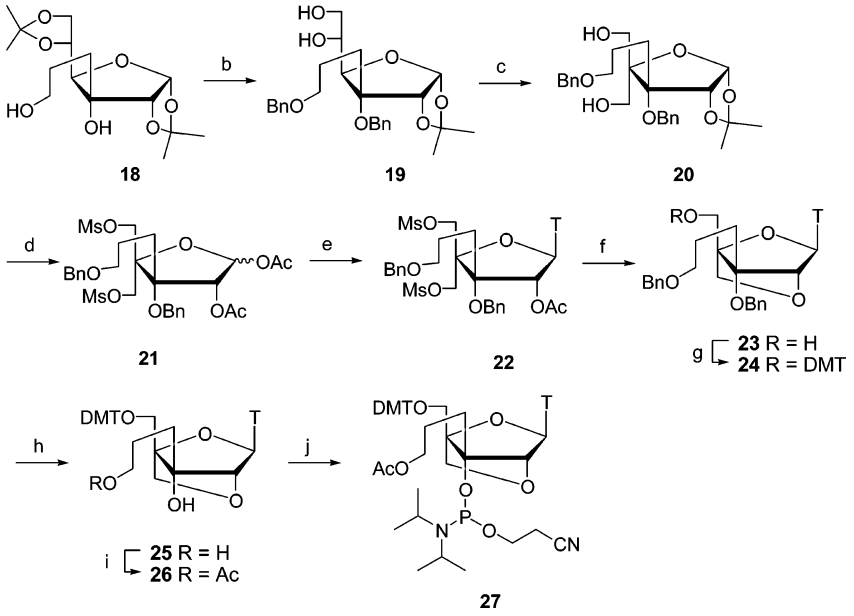
SCHEME 2[a]



[a] Key: (a) (i) BH_3Me_2S, THF, (ii) 2 M NaOH, H_2O_2, 70% (synthesis of furanose **18** from 3-*C*-allyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose;[22] (b) (i) NaH, BnBr, DMF, (ii) AcOH/H_2O (8:2), 79%; (c) (i) $NaIO_4$, H_2O, 1,4-dioxane, THF, (ii) HCHO, 1 M NaOH, 1,4-dioxane, 85%; (d) (i) MsCl, pyridine, (ii) TFA/H_2O (8:2), (iii) Ac_2O, pyridine, 87%; (e) thymine, *N*,*O*-(bistrimethylsilyl)acetamide, TMS-triflate, CH_3CN, 93%; (f) H_2O, KOH, 1,4-dioxane, 66%; (g) DMTCl, pyridine, 90%; (h) Pd/C, $HCOONH_4$, MeOH, 74%; (i) Ac_2O, pyridine, 90%; (j) $(^iPr)_2NP(Cl)OCH_2CH_2CN$, $(^iPr)_2NEt$, CH_2Cl_2, 64%. T = thymin-1-yl.

by tosylation of the hydroxy group of the 4′-*C*-hydroxymethyl substituent would it be possible to obtain LNA type derivatives. Treatment of **11** with NaH in DMF did indeed confirm the site of tosylation as two singlets gradually appeared in the ^1H NMR spectrum as the reaction progressed, and after 12 h the 3′-*C*-allyl LNA derivative of **11** was isolated (data not shown).

DMT (4,4′-dimethoxytrityl) protection of the remaining primary hydroxy group of nucleoside **11** was accomplished by reaction with 4,4′-dimethoxytrityl chloride (DMTCl) in anhydrous pyridine furnishing nucleoside **12** in 74% yield. For the synthesis of the 3′-C-branched LNA monomer, compound **13** was treated with NaH in DMF to afford cyclization, which proceeded smoothly to give the bicyclic nucleoside **13** in 96% yield. The allyl moiety was cleaved in an oxidative manner, taking advantage of an OsO_4-catalyzed dihydroxylation and subsequent cleavage of the newly formed diol system with $NaIO_4$. The resulting aldehyde was finally reduced with $NaBH_4$ to give the hydroxyethyl branched LNA nucleoside **14** in only 38% yield. The rather low yield in this reaction is comparable to low yields reported for similar reactions on related compounds.[26,27] The benzyl protective group was subsequently removed by treatment of nucleoside **14** with ammonium formate and Pd/C in refluxing methanol to give nucleoside diol **15** in 83% yield. The primary hydroxy group of **15** was selectively protected as its levulinic ester by reaction with levulinic anhydride in pyridine[28,29] giving the nucleoside **16** in 88% yield (Scheme 1). Subsequent phosphitylation using 2-cyanoethyl *N*,*N*-(diisopropyl)phosphoramidochloridite and *N*,*N*-(diisopropyl)ethylamine in dichloromethane afforded the desired LNA-type phosphoramidite building block **17** (41% yield) that was used for incorporation of the 3′-*C*-hydroxyethyl LNA-type monomer **3** (Figure 1, R = OH) into ONs.[18]

However, two facts prompted us to engage in the synthesis of the 3′-propyl derivatized phosphoramidites **27** and **38** (Schemes 2 and 3). One was the low overall yield of the synthetic route toward phosphoramidite **17**, in particular the very low yield of the step leading to the 3′-*C*-hydroxyethyl nucleoside **15**. The second was the somewhat disappointing lack of stabilizing effect of 3′-*C*-hydroxyethyl LNA-type monomer **3** when evaluated in a duplex toward a DNA complement in the preliminary study.[18]

Synthesis of 3′-*C*-Hydroxypropyl LNA-Type Nucleosides. 3-*C*-Allyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose[22] was converted into the corresponding 3-*C*-(3-hydroxy)propyl derivative by hydroboration using BH_3Me_2S in THF followed by oxidation with a mixture of NaOH and H_2O_2 giving furanose **18** in 70% yield. Benzylation of the primary and the tertiary hydroxyl groups using sodium hydride and benzyl bromide in DMF, followed by the selective removal of the 5,6-*O*-isopropylidene protective group using 80% aqueous acetic acid at rt for 45 h, afforded the 5,6-diol **19** in 79% yield. The resulting 5,6-diol system was oxidatively cleaved using $NaIO_4$ in a mixture of 1,4-dioxane, THF, and H_2O followed by an aldol condensation between the resulting aldehyde and formaldehyde and reduction under in situ Cannizzaro reaction conditions to furnish the diol **20** in 85% yield. Mesylation of the two primary hydroxyl groups

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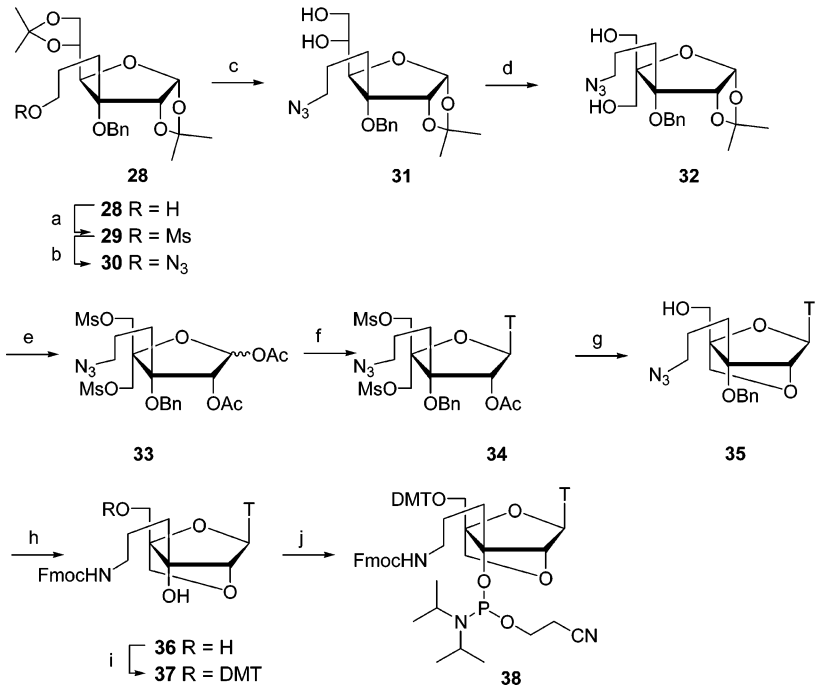
SCHEME 3[a]



[a] Key: (a) MsCl, pyridine, 79%; (b) NaN_3, DMF, 84%; (c) $AcOH/H_2O$ (8:2), 82%; (d) (i) $NaIO_4$, 1,4-dioxane, H_2O, THF, (ii) HCHO, 1 M NaOH, 1,4-dioxane, 89%; (e) (i) MsCl, pyridine, (ii) TFA/H_2O (8:2), (iii) Ac_2O, pyridine, 67%; (f) thymine, *N*,*O*-bis(trimethylsilyl)acetamide, TMS-triflate, CH_3CN, reflux, 75%; (g) KOH, H_2O, 1,4-dioxane, reflux, 63%; (h) (i) H_2, $Pd(OH)_2/C$, MeOH, (ii) Fmoc-Cl, pyridine, 51%; (i) DMTCl, pyridine, 68%; (j) $(^iPr)_2NP(Cl)OCH_2CH_2CN$, $(^iPr)_2NEt$, CH_2Cl_2, 87%. T = thymin-1-yl.

using methanesulfonyl chloride in pyridine followed by removal of the 1,2-*O*-isopropylidene protective group with 80% trifluoroacetic acid and acetylation of the resulting two hydroxyl groups using acetic anhydride in pyridine gave the anomeric mixture **21** in 87% yield, which was used as a glycosyl donor in a modified Vorbrüggen condensation reaction. Thus, the anomeric mixture **21** was stirred under reflux for 23 h with silylated thymine and TMS-triflate to give the monoacetylated nucleoside **22** in 93% yield. The nucleoside was obtained solely as the β-anomer due to anchimeric assistance from the 2-*O*-acetyl group. The acetylated nucleoside was converted into LNA-type nucleoside **23** in 66% yield by treatment of **22** with aqueous potassium hydroxide and 1,4-dioxane with heating under reflux as described previously for other LNA-type nucleosides.[30] This reaction involves hydrolytic removal of the 2′-*O*-acetyl group, cyclization by intramolecular nucleophilic attack of the 2′-hydroxy group, and removal of the 5′-*O*-mesyl group. The primary hydroxyl group of nucleoside **23** was DMT protected as described for synthesis of nucleoside **13** affording nucleoside **24** in 90% yield. Debenzylation using ammonium formate and Pd/C in methanol gave diol **25** in 74% yield, and subsequent selective acetylation of the primary hydroxy group with acetic anhydride in pyridine afforded the monoacetylated nucleoside **26** in 90% yield. Finally, standard phosphitylation using 2-cyanoethyl *N*,*N*-(diisopropyl)phosphoramidochloridite and *N*,*N*-(diisopropyl)-ethylamine in dichloromethane afforded the desired 3′-*C*-acetoxypropyl LNA-type phosphoramidite building block **27** in 64% yield suitable for incorporation of monomer **X** (Table 1) into ONs (Scheme 2).

The synthesis of the protected 3′-*C*-hydroxyethyl LNA-type phosphoramidite **17** (Scheme 1) proceeded in only 1.3% overall yield, whereas the synthesis of the protected 3′-*C*-hydroxypropyl phosphoramidite derivative **27** (Scheme 2) proceeded in 10% overall yield. As the former synthesis furthermore involves more synthetic steps, the latter offers significant advantages toward synthesis of 3′-C-branched LNA-type nucleosides, and it therefore formed the basis for various synthetic strategies toward the corresponding 3′-*C*-aminopropyl LNA-type nucleosides. However, attempts at introducing various N-nucleophiles at activated 3′-*C*-hydroxypropyl nucleosides (both protected 4′-*C*-hydroxymethyl derivatives and bicyclic LNA-type derivatives) were unsuccessful in our hands, and we therefore decided to introduce an N-nucleophile very early in the synthetic route (Scheme 3).

Synthesis of 3′-*C*-Aminopropyl LNA-Type Nucleosides. In general, a reaction sequence quite similar to the one used for synthesis of the 3′-*C*-hydroxypropyl LNA-type nucleosides (see Scheme 2) was used. Thus, starting from furanose **6**, hydroboration using BH_3Me_2S in THF and oxidation using a mixture of aq NaOH and H_2O_2 afforded the primary alcohol **28** in 46% yield. Mesylation of the primary hydroxy group to give compound **29** in 79% yield followed by introduction of an azido group using NaN_3 in DMF at 100 °C yielded azido compound **30** in 84% yield. The introduction of the azido group was verified by IR spectroscopy. Selective removal of the 5,6-*O*-isopropylidene protective group to give 5,6-diol **31** in 82% yield and subsequent oxidative cleavage

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using $NaIO_4$ in a mixture of 1,4-dioxane, THF, and H_2O followed by a Cannizzaro reaction afforded the diol **32** in 89% yield. Mesylation of the two primary hydroxyl groups, removal of the 1,2-*O*-isopropylidene protective group, and acetylation of the resulting two hydroxyl groups furnished the anomeric mixture **33** in 67% yield. Coupling between **33** and thymine using TMS-triflate as Lewis acid and *N*,*O*-bis(trimethylsilyl)acetamide as the silylating agent provided nucleoside **34** in 75% yield. Deacetylation of the 2′-*O*-acetyl group and cyclization by an intramolecular nucleophilic attack of the resulting 2′-hydroxy group with concomitant demesylation[30] was performed in a mixture of aqueous potassium hydroxide and 1,4-dioxane at reflux affording LNA-type nucleoside **35** in 63% yield. Reduction of the azide and debenzylation with H_2 and $Pd(OH)_2$/C in methanol followed by Fmoc protection of the primary amino group with Fmoc-Cl in pyridine afforded the Fmoc-protected 3′-*C*-aminopropyl LNA nucleoside **36** in 51% yield. Using standard methods, the primary hydroxy group of nucleoside **36** was DMT protected to give nucleoside **37** (68% yield) which was followed by phosphitylation in 87% yield to furnish the desired Fmoc-protected 3′-*C*-aminopropyl phosphoramidite building block **38** (Scheme 3).

LNA ON Synthesis and Thermal Denaturation Studies. To test the properties of ONs containing the novel 3′-C-branched LNA monomers **X**, **Y**, and **Z**, we efficiently synthesized **LNA-1**, **LNA-2**, and **LNA-3** (Table 1) using the phosphoramidite approach on an automated DNA synthesizer (see the Experimental Section for details). In addition, **LNA-3**, while still attached at the solid support, was also used for synthesis of **LNA-4** employing an on-column conjugation approach involving selective removal of the Fmoc group and subsequent reaction with Fmoc-protected glycine and HBTU in DMF following a procedure (see Experimental Section for details) published for synthesis of 5′-end-conjugated ONs.[31] The composition of the synthesized LNA ONs was verified by MALDI-MS and the purity (>80%) by capillary gel electrophoresis/reversed-phase HPLC. Hybridization data of these modified LNA ONs toward complementary single stranded DNA and RNA are shown in Table 1. For comparison, data of the unmodified DNA reference (**DNA-1**) are also shown.

Comparison between the reference **DNA-1** and **LNA-1**, containing three 3′-*C*-hydroxypropyl LNA-type monomers **X** and six DNA monomers under medium salt conditions, confirm earlier results with the 3′-*C*-hydroxyethyl LNA-type monomer (**3**, R = OH, Figure 1),[18] namely that the increase in stability expected by the introduction of the bicyclic LNA scaffold is counterbalanced by the apparently unfavorable introduction of the 3′-*C*-alkyl substituent. It appears that a 3′-*C*-alkyl substituent is more unfavorable with respect to hybridization when attached at an nucleotide adopting a 3′-*endo* type (N-type) furanose conformation than when attached at a flexible DNA-type monomer.[15−18] On the contrary, a clear stabilizing effect, especially toward complementary RNA, is observed for **LNA-2** containing three 3′-*C*-aminopropyl LNA-type monomers **Y**. This effect is confirmed against the DNA complement for the singly modified **LNA-3**. The *N*-glycyl monomer **Z** was incorporated once (**LNA-4**) allowing direct comparison with **LNA-3**. It is observed that the exact positioning of the primary amino group plays an important role as monomer **Z**, contrary to monomer **Y**, has no stability-increasing effect.

To study further the affinity-enhancing effect of 3′-*C*-aminopropyl LNA-type monomer **Y**, thermal denaturation studies under low salt conditions were conducted. These data strongly suggest that monomer **Y** is protonated at the conditions applied as its stability increasing effect, relative to monomer T (**DNA-1**) and monomer **X** (**LNA-1**), is further enhanced (see data for **LNA-2** and **LNA-3**). The data obtained for **LNA-4** indicate in general a loss of the positive effect observed for monomer **Y**, possibly caused by inappropriate positioning of the primary amino group in the 3′-*C*-branch of monomer **Z**.

A general trend is that introduction of the 3′-C-branched LNA-type monomers, like unmodified LNA monomers,[6−9] increases duplex stability more toward complementary RNA than DNA. It is therefore clear that furanose ring conformation plays a role, but to what degree the stability increasing effect of introducing a primary amino group, as clearly shown for monomer **Y**, is mainly due to reduced electrostatic repulsion, the formation of specific ion pairs between the protonated amino groups and the phosphate backbone, or the formation of stabilizing hydrogen bonds, remains to be elucidated.

Conclusion

Synthetic routes toward the three protected 3′-C-branched LNA-type phosphoramidite building blocks **17**, **27,** and **38**, containing furanose rings locked in an N-type conformation, have been developed starting from a known 3-*C*-allyl allofuranose derivative. The branching alkyl chain was introduced before condensation with the nucleobase, and conversion of a hydroxypropyl branch into an aminopropyl branch proved efficient only at a stage prior to condensation with the thymine nucleobase. The synthesis developed for the 3′-*C*-hydroxypropyl derivatives is clearly superior to that of the corresponding 3′-*C*-hydroxyethyl derivative. The novel phosphoramidites **27** and **38** were applied on an automated DNA synthesizer leading to the introduction of three novel 3′-*C*-branched LNA-type monomers **X**, **Y**, and **Z** into oligodeoxynucleotides. A duplex stabilizing effect of introducing 3′-*C*-aminopropyl-LNA monomer **Y**, relative to 3′-*C*-hydroxypropyl-LNA monomer **X**, was observed, especially at low salt conditions. This indicates that the primary amino group of monomer **Y** is protonated under the hybridization conditions applied and that the positioning of a positive charged group in the major groove has a stabilizing effect. Monomer **Y** was by an on-column conjugation method further functionalized by a glycyl unit to give monomer **Z** that showed a smaller stabilizing effect than monomer **Y**. This points to the importance of the three-dimensional positioning of a positively charged amino group, i.e., Ångström-scale structural control, if a strong duplex stabilization is aimed for.

Experimental Section

3-*C*-Allyl-3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (5). To a stirred solution of 3-*C*-allyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose[22] (5.33 g, 17.8 mmol) in

(31) Mokhir, A. A.; Tetzlaff, C. N.; Herzberger, S.; Mosbacher, A.; Richert, C. *J. Comb. Chem.* **2001**, *3*, 374.

anhydrous DMF (20 mL) at rt was added NaH (1.06 g, 26.61 mmol). Benzyl bromide (2.8 mL, 25.8 mmol) was added after 30 min, and the reaction mixture was stirred for 3 h. H_2O (10 mL) was added, and the resulting mixture was evaporated to dryness. The residue was dissolved in EtOAc (150 mL), washed successively with H_2O (70 mL) and NaCl (70 mL), dried (Na_2SO_4), filtered, and evaporated to dryness. Column chromatography (20% EtOAc/petroleum ether) afforded furanose **5** (6.60 g, 16.8 mmol, 95%) as a white solid material: R_f 0.56 (EtOAc/petroleum ether, 2:5 (v/v)); ^{1}H NMR ($CDCl_3$) 7.41−7.21 (m, 5H, Bn), 6.09−5.93 (m, 1H, CH_2CHCH_2−), 5.63 (d, J 3.7, 1H, 1-H), 5.20−5.13 (m, 2H, CH_2CHCH_2−), 4.85 (d, J = 11.2 Hz, 1H, Bn), 4.72 (d, J = 11.2 Hz, 1H, Bn), 4.49 (d, J = 3.7 Hz, 1H, 2-H), 4.25−3.94 (m, 4H, 4-H, 5-H, 6-H), 2.69 (dd, J = 6.8, 14.9 Hz, 1H, CH_2CHCH_2−), 2.41 (dd, J = 7.3,14.9 Hz, 1H, CH_2CHCH_2−), 1.59, 1.39, 1.35 and 1.34 (4 × s, 4 × 3H, 4 × CH_3-isopropylidene); ^{13}C NMR ($CDCl_3$) 139.3, 132.7, 128.1, 127.3, 118.7, 112.7, 109.6, 103.5, 83.7, 83.0, 81.3, 73.1, 69.1, 66.9, 35.9, 27.0, 26.6, 25.4; FAB-MS m/z 391 ([M + 1]$^+$). Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.99; H, 7.49.

3-*C*-Allyl-3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (6). A solution of furanose **5** (6.90 g, 17.8 mmol) in 80% aq acetic acid (100 mL) and methanol (1 mL) was stirred for 18 h. The solvent was removed under reduced pressure, and the residue was coevaporated with toluene (2 × 20 mL). The residue was dissolved in EtOAc (150 mL), and washing was performed using satd aq $NaHCO_3$ (50 mL). The organic phase was evaporated to dryness, the residue was dissolved in THF (40 mL), and $NaIO_4$ (5.58 g, 26.1 mmol) dissolved in H_2O (40 mL) was added. The reaction was stirred for 1 h at rt, whereupon H_2O (20 mL) was added. The resulting mixture was extracted with dichloromethane (2 × 100 mL). The organic phase was washed with NaCl (20 mL) and evaporated to dryness. The residue was dissolved in THF (40 mL), formaldehyde (5 mL, 37% solution (w/w) in methanol) and NaOH (2 M, 20 mL) were added, and the reaction mixture was stirred for 12 h at rt. The mixture was cooled to 0 °C, $NaBH_4$ (1.00 g, 26 mmol) was added, and stirring was continued at rt for 1 h. The mixture was evaporated to half volume under reduced pressure and diluted with H_2O. The extraction was performed using EtOAc (2 × 100 mL), and the separated organic phase was washed with satd aq $NaHCO_3$ (2 × 30 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography (EtOAc/petroleum ether, 2:5 (v/v)) to give furanose **6** as a pale yellow oil (3.69 g, 10.6 mmol, 59%): R_f 0.18 (EtOAc/petroleum ether, 2:5 (v/v)); ^{1}H NMR ($CDCl_3$) 7.39−7.15 (m, 5H, Bn), 5.97−5.91 (m, 1H, CH_2CHCH_2−), 5.72 (d, J = 4.2 Hz, 1H, 1-H), 5.29−5.20 (m, 2H, CH_2CHCH_2−), 4.72 (d, J = 10.4 Hz, 1H, Bn), 4.65 (d, J = 10.4, 1H, Bn), 4.53 (d, J = 4.2 Hz, 1H, 2-H), 4.10−3.87 (m, 4H, 4-H, 5-H, 6-H), 2.76 (dd, J = 8.1, 15.0 Hz, 1H, CH_2CHCH_2−), 2.65 (dd, J = 6.1, 15.0 Hz, 1H, CH_2CHCH_2−), 1.65 and 1.33 (2 × s, 2 × 3H, 2 × CH_3-isopropylidene); ^{13}C NMR ($CDCl_3$) 138.0, 131.9, 129.0, 128.4, 128.3, 128.2, 127.7, 127.5, 127.2, 125.3, 119.6, 112.7, 104.2, 87.4, 86.0, 83.1, 67.2, 63.9, 63.5, 36.9, 26.2, 25.7; FAB-MS m/z 351 ([M + 1]$^+$). Anal. Calcd for $C_{19}H_{26}O_6$: C, 64.88; H, 7.40. Found: C, 65.13; H, 7.48.

4-*C*-Acetoxymethyl-5-*O*-acetyl-3-*C*-allyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (7). To a solution of furanose **6** (3.30 g, 9.44 mmol) in anhydrous pyridine (13 mL) were added acetic anhydride (13.0 mL, 138 mmol) and a catalytic amount of DMAP, and the reaction mixture was stirred for 12 h at rt. Ice-cold H_2O (300 mL) was added, and extraction was performed using dichloromethane (2 × 50 mL). The organic phase was evaporated to dryness, and the residue was purified by column chromatography (EtOAc/petroleum ether, 1:1 (v/v)) affording furanose **7** as a clear oil (3.50 g) that was used in the next step without further purification: R_f 0.67 (EtOAc/petroleum ether, 1:1 (v/v)); ^{13}C NMR ($CDCl_3$) 170.4, 170.0, 138.0, 131.1, 128.1, 127.2, 126.7, 119.5, 112.9, 103.9, 86.1, 85.2, 83.1, 66.6, 63.2, 62.2, 36.2, 26.1, 25.7, 20.9, 20.8; FAB-MS m/z 435 ([M + 1]$^+$).

4-*C*-Acetoxymethyl-3-*C*-allyl-3-*O*-benzyl-1,2,5-tri-*O*-acetyl-D-*erythro*-pentofuranose (8). A solution of the product of the preceding reaction (furanose **7**, 5.50 g) in 80% aq acetic acid (36 mL) was stirred for 3 h at 90 °C. The reaction mixture was evaporated to dryness and coevaporated with ethanol (2 × 30 mL), anhydrous toluene (2 × 30 mL), and anhydrous pyridine (30 mL), and the residue was dissolved in a mixture of anhydrous pyridine (13 mL) and acetic anhydride (13.0 mL, 138 mmol). A catalytic amount of DMAP was added, and the reaction mixture was stirred at rt for 12 h. Ice-cold H_2O (300 mL) was added, and the resulting mixture was extracted with dichloromethane (2 × 200 mL). The combined organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (EtOAc/petroleum ether, 1:10−1:5 (v/v)) to give the anomeric mixture **8** as a clear oil (3.38 g) that was used in the next step without further purification: R_f 0.67 (EtOAc/petroleum ether, 1:1 (v/v)); ^{13}C NMR ($CDCl_3$) 170.4, 169.8, 169.3, 169.1, 137.7, 131.6, 131.3, 128.6, 128.5, 128.4, 128.3, 127.6, 127.5, 126.9, 126.7, 119.8, 119.6, 98.1, 92.8, 88.3, 87.6, 84.0, 82.6, 79.6, 76.0, 66.7, 64.7, 63.5, 63.3, 62.4, 34.8, 34.7, 21.0, 21.0, 20.8, 20.7; FAB-MS m/z 419 ([M − AcO]$^+$).

1-(4-*C*-Acetoxymethyl-3-*C*-allyl-3-*O*-benzyl-2,5-di-*O*-acetyl-β-D-*erythro*-pentofuranosyl)thymine (9). The product of the preceding reaction (anomeric mixture **8**, 4.76 g) was coevaporated with anhydrous acetonitrile (2 × 30 mL) and dissolved in anhydrous acetonitrile (100 mL). Thymine (2.72 g, 21.6 mmol) and *N*,*O*-(bistrimethyl)acetamide (18.6 mL, 75 mmol) were added, and the reaction mixture was heated under reflux with stirring for 1 h. The reaction mixture was cooled to 0 °C whereupon trimethylsilyl triflate (5.0 mL, 27 mmol) was added. After the resulting mixture was heated to 65 °C, stirring was continued for 2 h. Saturated aq $NaHCO_3$ (100 mL) was added followed by extraction using dichloromethane (3 × 70 mL). The combined organic phase was washed with satd aq NaCl (65 mL), dried (Na_2SO_4), and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (EtOAc/petroleum ether, 2:3−1:1 (v/v)) to give nucleoside **9** as a white foam (3.60 g, 6.70 mmol, 63% calculated from furanose **7**): R_f 0.23 (EtOAc/petroleum ether, 1:1; ^{1}H NMR ($CDCl_3$) 9.42 (s, 1H, NH), 7.40 (s, 1H, 6-H), 7.38−7.28 (m, 5H, Bn), 6.39 (d, J = 8.1 Hz, 1H, 1′-H), 5.88−5.86 (m, 1H, CH_2CHCH_2−), 5.57 (d, J = 8.1 Hz, 1H, 2′-H), 5.27−5.23 (m, 2H, CH_2CHCH_2−), 5.06 (d, J = 11.0 Hz, 1H, Bn), 4.72 (d, J = 11.2 Hz, 1H, Bn), 4.55−4.30 (m, 4H, 5′-H, 5″-H), 3.00 (dd, J = 5.5, 16.5 Hz, 1H, CH_2CHCH_2−), 2.47 (dd, J = 8.2, 16.7 Hz, 1H, CH_2CHCH_2−), 2.22, 2.13, and 2.04 (3 × s, 3 × 3H, 3 × $COCH_3$), 1.94 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) 170.2, 169.9 and 169.5 (CO), 163.6 (C-4), 150.8 (C-2), 137.7 (Bn), 134.8 (C-6), 130.8 (CH_2CHCH_2−), 128.5, 128.4, 128.3, 127.8, 127.7, 127.4, 127.2, CH_2CHCH_2-, 111.4 (C-5), 86.9 (C-4′), 83.9 (C-1′), 83.3 (C-3′), 77.9 (C-2′), 67.0 (Bn), 64.3 and 64.1 (C-5′, C-5″), 39.6 (CH_2CHCH_2−), 21.0, 20.9, 20.8, 20.8 ($COCH_3$), 12.7 (CH_3); FAB-MS m/z 419 ([M − T]$^+$). Anal. Calcd for $C_{21}H_{26}N_2O_7$: C, 59.55; H, 5.92; N, 5.14. Found: C, 59.34; H, 6.14; N, 5.03.

1-(3-*C*-Allyl-3-*O*-benzyl-4-*C*-hydroxymethyl-β-D-*erythro*-pentofuranosyl)thymine (10). To a stirred solution of nucleoside **9** (1.37 g, 2.52 mmol) in methanol (17 mL) at rt was added NaOMe (0.86, 15 mmol), and stirring was continued for 1 h. The reaction mixture was neutralized by addition of a 2 M solution of HCl in dioxane, and the resulting mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography (methanol/dichloromethane, 3:100−1:20, v/v) affording nucleoside **10** (1.85 g, 4.43 mmol, 81%) as a white solid material: R_f 0.36 (methanol/dichloromethane, 1:9 (v/v)); ^{1}H NMR (DMSO-d_6) 11.25 (s, 1H, NH), 7.99 (s, 1H, 6-H), 7.35−7.23 (m, 5H, Bn), 5.99−5.97 (m, 2H, 1′-H, CH_2CHCH_2−), 5.67 (d, J = 5.8 Hz, 1H, 2′-OH), 5.44 (t, J = 3.7 Hz, 1H, primary OH), 5.23−5.09 (m, 3H, CH_2CHCH_2−, $^1/_2CH_2$), 4.74 (d, J = 12.2, 1H, $^1/_2CH_2$), 4.54 (t, J =

5.6 Hz, 1H, primary OH), 4.36 (dd, J = 5.8, 8.2 Hz, 1H, 2′-H), 3.81−3.45 (m, 4H, 2 × CH_2), 3.08 (dd, J = 5.6, 16.2 Hz, 1H, CH_2CHCH_2−), 2.69 (dd, J = 8.0, 16.4 Hz, 1H, CH_2CHCH_2−), 1.78 (d, J = 1.0 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 163.7 (C-4), 151.3 (C-2), 139.6, 137.8 and 134.0 (Bn, C-6 and CH_2-$CHCH_2$−), 128.1, 126.9 and 126.6 (Bn), 117.8 (CH_2CHCH_2−), 109.4 (C-5), 90.2 (C-3′ or C-4′), 85.1 (C-1′), 83.5 (C-3′ or C-4′), 78.8 (C-2′), 65.6 (Bn), 63.0, 62.3 (C-5′, C-5″), 33.0 (CH_2CHCH_2−), 12.4 (CH_3); FAB-MS m/z 419 ([M + 1]$^+$). Anal. Calcd for $C_{21}H_{26}N_2O_7$·0.25 H_2O: C, 59.64; H, 6.32; N, 6.62. Found: C, 59.35; H, 6.32; N, 6.75.

1-(3-*C*-Allyl-3-*O*-benzyl-4-*C*-tosyloxymethyl-β-D-ribofuranosyl)thymine (11). Nucleoside **10** (1.69 g, 4.04 mmol) was coevaporated with anhydrous toluene (2 × 10 mL) and dissolved in anhydrous pyridine (42 mL). The mixture was cooled to 0 °C under stirring whereupon a solution of *p*-toluenesulfonyl chloride (895 mg, 4.71 mmol) in anhydrous pyridine (4 mL) was added slowly during 1 h. After an additional 30 min, the reaction mixture was allowed to warm to rt, and the reaction mixture was stirred for 22 h. H_2O (60 mL) was added, and extraction was performed using dichloromethane (3 × 100 mL). The combined organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (methanol/dichloromethane, 1:50 (v/v)) affording nucleoside **11** as a white foam (1.62 g, 2.83 mmol, 70%): R_f 0.41 (methanol/dichloromethane, 1:9 (v/v)); ^{1}H NMR ($CDCl_3$) 10.05 (br s, 1H, NH), 8.59 (s, 1H, 6-H), 7.75−7.18 (m, 9H, Ts and Bn), 5.91−5.85 (m, 1H, CH_2CHCH_2−), 5.40−3.74 (m, 12 H, 5′-H, 5″-H, Bn, 1′-H, 2′-H, CH_2CHCH_2−, 5′-OH and 2′-OH), 3.16−3.11 (m, 1H, CH_2CHCH_2−), 2.82−2.77 (m, 1H, CH_2CHCH_2−), 2.40 (s, 3H, Ts), 1.77 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) 164.4 (C-4), 150.7 (C-2), 149.0−123.8 (Bn, Ts, CH_2CHCH_2−, C-6), 119.3 (CH_2-$CHCH_2$−), 110.4 (C-5), 92.5, 88.4, 83.4 and 76.6 (C-1′, C-2′, C-3′ and C-4′), 70.1, 66.6 and 62.5 (C-5′, C-5″ and Bn), 33.4 (CH_2CHCH_2−), 21.6 (Ts), 12.8 (CH_3); FAB-MS m/z 573 ([M + 1]$^+$). Anal. Calcd for $C_{28}H_{32}N_2O_9S$·0.25H_2O: C, 58.27; H, 5.68; N, 4.85. Found: C, 58.25; H, 5.57; N, 4.75.

1-(3-*C*-Allyl-3-*O*-benzyl-4-*C*-tosyloxymethyl-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl)thymine (12). To a stirred solution of nucleoside **11** (1.60 g, 2.79 mmol) in anhydrous pyridine (6.4 mL) at rt was added DMTCl (1.59 g, 4.69 mmol). Stirring was continued for 42 h, whereupon methanol (6 mL) was added. The resulting mixture was evaporated to dryness under reduced pressure, and the residue was dissolved in dichloromethane (65 mL). Washing was performed using satd aq $NaHCO_3$ (2 × 50 mL), and the separated organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (methanol/dichloromethane/pyridine, 0:99.75:0.25−2:97.75:0.25 (v/v/v)) to give nucleoside **12** (1.81 g, 2.06 mmol, 74%) as a white foam: R_f 0.35 (methanol/dichloromethane, 1:19 (v/v)); ^{13}C NMR ($CDCl_3$) 163.7 (C-4), 158.6 (DMT), 150.8 (C-2), 149.1−123.7 (DMT, Bn, Ts, CH_2-$CHCH_2$−, C-6), 118.9 (CH_2CHCH_2−), 113.0 (DMT), 110.9 (C-5), 88.2, 87.2, 83.3 and 78.8 (C-1′, C-2′, C-3′ and C-4′), 85.5 (DMT), 70.4, 66.6 and 62.9 (C-5′, C-5″ and Bn), 55.2 (DMT), 33.1 (CH_2CHCH_2−), 21.6 (Ts), 10.9 (CH_3); FAB-MS m/z 874 ([M]$^+$). Anal. Calcd for $C_{49}H_{50}N_2O_{11}S$·0.5H_2O: C, 66.58; H, 5.82; N, 3.17. Found: C, 66.42; H, 5.77; N, 3.58.

(1*R*,3*R*,4*R*,7*S*)-7-Allyl-7-benzyloxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]-heptane (13). To a stirred solution of nucleoside **12** (2.79 g, 3.19 mmol) in anhydrous DMF (80 mL) at rt was added NaH (638 mg, 60% suspension, 16 mmol). After the mixture was stirred for 20 h, H_2O (6 mL followed after 5 min by additional 100 mL) was added, and the resulting mixture was extracted with EtOAc (2 × 250 mL). The combined organic phase was dried (Na_2SO_4), evaporated to dryness, and coevaporated with anhydrous toluene (2 × 40 mL). The residue was purified by column chromatography (pyridine/methanol/dichloromethane, 0.25:1.0:98.75 (v/v/v)) yielding the bicyclic nucleoside **13** (2.15 g, 3.06 mmol, 96%) as a white foam: R_f 0.41 (methanol/dichloromethane, 7:93 (v/v)); ^{1}H NMR ($CDCl_3$) 9.87 (s, 1H, NH), 7.50−5.52 (m, 19H, DMT, Bn, 6-H), 5.69−5.58 (m, 1H, CH_2CHCH_2−), 5.52 (s, 1H, 1′-H), 5.14 (s, 1H, 2′-H), 4.90 (dd, J = 10.1, 20.9 Hz, 2H, CH_2CHCH_2−), 4.57 (s, 2H, CH_2), 4.29 (d, J = 7.9 Hz, 1H, CH_2), 4.15 (d, J = 7.9, 1H, CH_2), 3.78 (s, 6H, DMT), 3.57 (d, J = 11.0, 1H, CH_2), 3.43 (d, J = 11.0, 1H, CH_2), 2.38−2.36 (m, 2H, CH_2CHCH_2−), 2.02 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) 163.7 (C-4), 158.6 (DMT), 145.0 (C-2), 144.4−127.0 (DMT, Bn, C-6, CH_2CHCH_2−), 118.2 (CH_2CHCH_2−), 113.2 (DMT), 110.4 (C-5), 90.7 (C-4′), 88.9 (C-1′), 86.6 (DMT), 83.4 (C-3′), 78.5 (C-2′), 74.7, 67.9 and 59.8 (Bn, C-5′, C-5″), 55.2 (DMT), 32.7 (CH_2CHCH_2−), 12.8 (CH_3); FAB-MS m/z 703 ([M +1]$^+$). Anal. Calcd for $C_{42}H_{42}N_2O_8$: C, 71.78; H, 6.02; N, 3.99. Found: C, 72.13; H, 6.36; N, 3.71.

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-1-(4,4′-dimethoxytrityl)-oxymethyl-7-(2-hydroxyethyl)- 3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (14). To a stirred solution of compound **13** (2.14 g, 3.04 mmol) in THF (20 mL) at rt was added first a solution of $NaIO_4$ (2.45 g, 11.5 mmol) in H_2O (20 mL) and a solution of OsO_4 (0.41 mL, 2.5% solution in *tert*-butyl alcohol, 28 μmol), and the reaction mixture was stirred for 24 h. Ethylene glycol (8 mL, 144 mmol) was added, and the resulting mixture was stirred for 15 min whereupon H_2O (41 mL) was added. Extraction was performed using EtOAc (400 mL), and the organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was dissolved in a mixture of THF (20 mL) and H_2O (20 mL), and $NaBH_4$ (266 mg, 6.00 mmol) was added. After being stirred at rt for 20 h, the reaction mixture was neutralized by addition of a 2 M solution of HCl in dioxane, and the mixture was extracted using EtOAc (350 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure to give a residue that was purified by column chromatography (methanol/dichloromethane/pyridine, 1:98.75:0.25 (v/v/v)) to give compound **14** (813 mg, 1.15 mmol, 38%) as a white foam: R_f 0.43 (methanol/dichloromethane, 1:9 (v/v)); ^{1}H NMR ($CDCl_3$) 10.15 (s, 1H, NH), 7.51−6.81 (m, 21H, DMT, Bn, 6-H), 5.50 and 5.49 (s, 2H, 1′-H and 2′-H), 4.84 (d, J = 11.4 Hz, 1H, CH_2), 4.71 (d, J = 11.2 Hz, 1H, CH_2), 4.15 (d, J = 7.9 Hz, 1H, CH_2), 4.10 (d, J = 7.9 Hz, 1H, CH_2), 3.78 (s, 6H, DMT), 3.74−3.70 (m, 2H, C3′-CH_2CH_2OH), 3.53 (d, J = 10.8 Hz, 1H, CH_2), 3.47 (d, J = 10.8 Hz, 1H, CH_2), 1.92 (s, 3H, CH_3), 1.84−1.69 (m, 2H, C3′-CH_2CH_2OH); ^{13}C NMR ($CDCl_3$) 164.2 (C-4), 158.5 (DMT), 150.8 (C-2), 144.4−125.2 (DMT, Bn, C-6), 113.1 (DMT), 110.5 (C-5), 90.7 (C-4′), 89.0 (C-1′), 86.4 (DMT), 82.7 (C-3′), 78.9 (C-2′), 73.4 (C3′-CH_2CH_2OH), 68.8, 59.8, 57.3 (Bn, C-5′ and C-5″), 55.1 (DMT), 31.4 (C3′-CH_2CH_2OH), 12.7 (CH_3); FAB-MS m/z 706.2 ([M]$^+$). Anal. Calcd for $C_{41}H_{42}N_2O_9$: C, 69.67; H, 5.99; N, 3.96. Found: C, 70.04; H, 6.03; N, 3.76.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityl)oxymethyl-7-hydroxy-7-(2-hydroxyethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo-[2.2.1]heptane (15). To a stirred solution of compound **14** (214 mg, 0.30 mmol) in anhydrous methanol (6 mL) at rt were added NH_4HCO_3 (100 mg, 1.59 mmol) and Pd/C (30 mg). The reaction mixture was stirred at 70 °C for 2.5 h, cooled to rt, filtered, and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (methanol/dichloromethane/pyridine, 1.0:98.75:0.25−4.0:95.75:0.25 (v/v/v)) to give the bicyclic nucleoside **15** (156 mg, 0.25 mmol, 83%) as a white foam: R_f 0.38 (methanol/dichloromethane, 1:9 (v/v)); ^{1}H NMR ($CDCl_3$) 10.74 (s, 1H, NH), 7.48−6.81 (m, 14H, DMT and 6-H), 5.46 (s, 1H, 1′-H), 5.17 (s, 1H, 2′-H), 4.30 (s, 1H, OH), 4.27 (s, 1H, OH), 4.37 (d, J = 8.2 Hz, 1H, CH_2), 4.25 (d, J = 8.2 Hz, 1H, CH_2), 3.88−3.82 (m, 1H, C3′-CH_2CH_2OH), 3.79 (s, 6H, DMT), 3.79−3.75 (m, 1H, C3′-CH_2CH_2OH), 3.55 (d, J = 10.8 Hz, $^1/_2CH_2$), 3.31 (d, J = 11.0 Hz, $^1/_2CH_2$), 1.86 (s, 3H, CH_3), 1.68−1.55 (m, 2H, C3′-CH_2CH_2OH); ^{13}C NMR ($CDCl_3$) 164.3 (C-4), 158.6 (DMT), 151.2 (C-2), 135.7−126.9 (DMT and C-6), 113.2 (DMT), 110.3 (C-5), 90.1 (C-4′), 88.3 (C-1′), 86.4 (DMT), 80.7 (C-2′), 78.2 (C-3′), 73.6 and 59.9 (C-5′ and C-5″), 56.8 (C3′-CH_2CH_2OH), 55.2 (DMT), 31.5 (C3′-CH_2CH_2-

OH), 12.9 (CH_3); FAB-MS *m/z* 627 ([M + 1]$^+$). Anal. Calcd for $C_{34}H_{36}N_2O_9 \cdot H_2O$: C, 66.22; H, 5.88; N, 4.54. Found: C, 66.37; H, 5.80; N, 4.54.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityl)oxymethyl-7-hydroxy-7-(2-levulinoyloxyethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (16). To a stirred solution of didyclohexyl carbodiimide (0.41 g, 2.0 mmol) in anhydrous dioxane (3.75 mL) at rt was added levulinic acid (0.41 mL, 4 mmol), and the reaction mixture was stirred for 16 h. The mixture was filtered, and 0.5 mL of the filtrate was added to a solution of nucleoside **15** (103 mg, 0.17 mmol) and DMAP (5 mg) in pyridine (2 mL). The reaction mixture was stirred at rt for 14 h whereupon H_2O (1 mL) was added. After the mixture was stirred for 5 min, satd aq $NaHCO_3$ (5 mL) was added. The resulting mixture was extracted with EtOAc (3 × 15 mL), and the combined organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (methanol/dichloromethane/pyridine, 1.0:98.75:0.25 (v/v/v)) to furnish the bicyclic nucleoside **16** (107 mg, 0.15 mmol, 88%) as a white foam: R_f 0.40 (methanol/dichloromethane, 7:93 (v/v)); ^{1}H NMR ($CDCl_3$) 8.61 (d, J = 1.6 Hz, 1H), 7.47−6.83 (m, 14H), 5.47 (s, 1H), 4.82 (s, 1H), 4.22 (m, 1H), 4.19 (d, 1H, J = 8.8 Hz), 4.05 (d, J = 8.8 Hz, 1H), 3.99−3.97 (m, 1H), 3.80 (s, 6H), 3.53 (d, J = 10.9 Hz, 1H), 3.38 (d, J = 10.9 Hz, 1H), 2.94 (s, 1H), 2.70−2.41 (m, 4H), 2.16 (s, 3H), 1.94 (d, J = 0.9 Hz), 1.82−1.70 (m, 2H); ^{13}C NMR ($CDCl_3$) 206.0, 172.6, 163.5, 158.6, 149.9, 144.3−123.6, 113.2, 110.4, 89.4, 87.9, 86.6, 80.1, 78.1, 72.9, 60.2, 59.3, 55.1, 37.7, 29.7, 27.8, 27.5, 12.8; FAB-MS *m/z* 715 ([M + 1]$^+$). Anal. Calcd for $C_{39}H_{42}N_2O_{11} \cdot {}^1/_2H_2O$: C, 64.72; H, 5.99; N, 3.87. Found: C, 64.63; H, 5.84; N, 4.11.

(1*R*,3*R*,4*R*,7*S*)-7-(2-Levulinoyloxy)ethyl-7-(2-cyanoethoxy)(*N*,*N*-(diisopropyl)amino)phosphinoxy-1-(4,4′-dimethoxytrityl)oxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo-[2.2.1]heptane (17). Nucleoside **16** (104 mg, 0.15 mmol) was coevaporated with anhydrous acetonitrile (3 × 1 mL) and dissolved in anhydrous dichloromethane (1 mL) at rt under stirring. Anhydrous *N*,*N*-(diisopropyl)ethylamine (0.6 mL, 3.5 mmol) and 2-cyanoethyl *N*,*N*-(diisopropyl)phosphoramidochloridite (0.15 mL, 0.54 mmol) were added, and the mixture was stirred for 12 h at rt. Additional 2-cyanoethyl *N*,*N*-(diisopropyl)phosphoramidochloridite (0.20 mL, 0.72 mmol) was added, and the mixture was stirred for another 6 h at rt. H_2O (1 mL) was added, and the resulting mixture was diluted with EtOAc (20 mL). Washing was performed using successively satd aq $NaHCO_3$ (3 × 2 mL) and NaCl (3 × 2 mL). The separated organic phase was dried (Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (Et_3N, EtOAc, petroleum ether, 1:9:10 (v/v/v)) afforded a yellow oil, which was dissolved in anhydrous toluene (1 mL) and precipitated by addition of this solution into ice-cold petroleum ether (20 mL) under vigorous stirring to give phosphoramidite **17** (0.06 mmol, 41%) as a pale yellow oil: R_f 0.57 (Et_3N, EtOAc, CH_2Cl_2, 2:9:9 (v/v/v)); ^{31}P NMR (DMSO-d_6) 146.42, 146.05.

1,2:5,6-Di-*O*-isopropylidene-3-*C*-(3-hydroxy)propyl-α-D-allofuranose (18). To a stirred ice-cold solution of 3-*C*-allyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose[22] (13.74 g, 45.70 mmol) in anhydrous THF (240 mL) was added dropwise a solution of $BH_3 \cdot Me_2S$ (2 M solution in diethyl ether, 69 mL, 0.138 mol). After being stirred for 3 h at rt, the solution was cooled to 0 °C, and a mixture of 2 M NaOH (69 mL) and 35% H_2O_2 (14 mL) was added dropwise. The resulting mixture was stirred for 16 h at rt and extracted with EtOAc (300 mL). The organic phase was washed with brine (100 mL + 200 mL). The combined aqueous phase was extracted using EtOAc (200 mL), and the combined organic phase was evaporated to dryness under reduced pressure and coevaporated with toluene (2 × 25 mL). The residue was purified by dry column vacuum chromatography (EtOAc/petroleum ether, 72:28−100:0 (v/v) methanol/EtOAc, 1:99−12:88 (v/v)) affording furanose **18** (10.18 g, 70%) as a white powder: R_f 0.17 (EtOAc/petroleum ether, 7:3 (v/v); ^{1}H NMR ($CDCl_3$) 5.70 (d, J = 3.8 Hz, 1H, 1-H), 4.38 (d, J = 3.8 Hz, 1H, 2-H), 4.18−4.07 (m, 2H, 6-H), 3.95−3.86 (m, 1H, 5-H), 3.80 (d, J = 8.2 Hz, 1H, 4-H), 3.75−3.68 (m, 2H, C3-$CH_2CH_2CH_2OH$), 2.84 (br s, 1H, OH), 2.08 (br s, 1H, OH), 1.94−1.81 (m, 2H, C3-$CH_2CH_2CH_2OH$), 1.77−1.66 (m, 2H, C3-$CH_2CH_2CH_2OH$), 1.59, 1.45, 1.36 (3 × s, 12H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) 112.7, 109.8 (2 × isopropylidine), 103.7 (C-1), 82.5 (C-4), 81.2 (C-2), 79.2, 73.3 (C-6), 68.0 (C-5), 63.0 (C3−$CH_2CH_2CH_2OH$), 28.2, 26.8, 26.8, 26.6, 26.4, 25.5 (C3-$CH_2CH_2CH_2OH$, 4 × CH_3); MALDI-HRMS *m/z* calcd 341.15707 [M + Na]$^+$, *m/z* found 341.15650. Anal. Calcd for $C_{15}H_{26}O_7$: C, 56.59; H, 8.23. Found: C, 56.61; H, 8.23.

3-*O*-Benzyl-3-*C*-(3-benzyloxy)propyl-1,2-*O*-isopropylidene-α-D-allofuranose (19). To a stirred ice-cold solution of furanose **18** (10.69 g, 33.60 mmol) in anhydrous DMF (75 mL) was added a 60% oil suspension of NaH (4.07 g, 0.10 mol). After 3 h, benzyl bromide (16 mL, 0.13 mol) was added dropwise, and the reaction mixture was stirred for 3 h at rt. Ice-cold H_2O (50 mL) was added whereupon extraction was performed using EtOAc (3 × 100 mL). The combined organic phase was washed successively with brine (70 mL), H_2O (70 mL), and saturated aqueous $NaHCO_3$ (70 mL). The combined aqueous phase was extracted with EtOAc (50 mL), and the combined organic phase was evaporated to dryness under reduced pressure and coevaporated with toluene (3 × 25 mL). The residue was dissolved in AcOH/H_2O (8:2 (v/v), 250 mL) and allowed to stir for 45 h at rt before the mixture was evaporated to dryness under reduced pressure. The residue was extracted with EtOAc (200 mL) and the organic phase washed with H_2O (2 × 100 mL). The organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene (3 × 25 mL). The residue was purified by dry column vacuum chromatography (EtOAc/petroleum ether, 9:11−3:1 (v/v)) to give furanose **19** (12.16 g, 79%) as a clear oil: R_f 0.40 (EtOAc/petroleum ether, 7:3 (v/v)); ^{1}H NMR ($CDCl_3$) 7.38−7.25 (m, 10H, Bn), 5.69 (d, J = 3.7 Hz, 1H, 1-H), 4.68 (dd, J = 16.5, 10.6 Hz, 2H, CH_2Ph), 4.51 (s, 2H, CH_2Ph), 4.46 (d, J = 3.7 Hz, 1H, 2-H), 4.09 (d, J = 9.3 Hz, 1H, 4-H) 3.90−3.64 (m, 3H, 5-H, 6-H), 3.50 (t, 1H, C3-CH_2-CH_2CH_2OH), 2.76 (br s, 1H, OH), 2.15 (br s, 1H, OH), 2.04−1.74 (m, 4H, C3-$CH_2CH_2CH_2OH$), 1.60, 1.35 (2 × s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) 138.4, 138.1, 128.6, 128.5, 127.8, 127.7, 113.2, 104.2, 85.1, 82.5, 79.1, 73.2, 70.4, 69.7, 67.6, 64.9, 27.9, 26.9, 26.7, 24.0. MALDI-HRMS *m/z* calcd 481.21967 [M + Na]$^+$, *m/z* found 481.21830. Anal. Calcd for $C_{26}H_{34}O_7$: C, 68.10; H, 7.47. Found: C, 67.73; H, 7.42.

3-*O*-Benzyl-3-*C*-(3-benzyloxy)propyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (20). To a stirred ice-cold solution of $NaIO_4$ (1.67 g, 7.8 mmol) in H_2O/1,4-dioxane (2:1 (v/v), 90 mL) at rt was added dropwise furanose **19** (1.93 g, 4.2 mmol) dissolved in THF (25 mL). After 3 h, ethylene glycol (1.5 mL) was added, the resulting mixture was filtered, the filtering funnel was flushed with EtOAc (2 × 50 mL), and the filtrate extracted with EtOAc (2 × 100 mL). The combined organic phase was evaporated to dryness under reduced pressure and the residue dissolved in 1,4-dioxane (50 mL). Formaldehyde (0.95 mL, 37% aq solution (w/w), 12.9 mmol) and 1 M NaOH (50 mL, 50.0 mmol) were added, and the resulting mixture was stirred for 16 h at rt. A 17.45 M aqueous solution of formic acid was added to neutralize the mixture followed by evaporation to dryness under reduced pressure. To the residue was added EtOAc (2 × 100 mL), and the combined organic phase was evaporated to dryness under reduced pressure and coevaporated with toluene (50 mL) to give a residue that was purified by dry column vacuum chromatography (EtOAc/petroleum ether, 6:4−7:3 (v/v)) yielding furanose **20** (1.64 g, 85%) as a white powder: R_f 0.35 (EtOAc/petroleum ether, 7:3 (v/v)); ^{1}H NMR ($CDCl_3$) 7.35−7.24 (m, 10H, Bn), 5.76 (d, 1H, J = 4.1 Hz, 1-H), 4.67 (d, J = 10.5 Hz, 1H, CH_2Ph), 4.60 (d, J = 10.5 Hz, 1H, CH_2Ph), 4.51 (s, 2H, CH_2Ph), 4.50 (s, 1H, 2-H), 4.12 (s, 2H, 5-H), 3.87−3.80 (m, 2H, 5′-H), 3.51 (t, 2H, C3−$CH_2CH_2CH_2OH$), 2.66 (br s, 1H,

OH), 2.54 (br s, 1H, OH), 2.13−1.72 (m, 4H, C3−CH_2CH_2CH_2-OH), 1.64, 1.31 (2 × s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) 138.5, 138.2, 128.5, 127.8, 127.8, 127.7, 127.7, 127.6, 112.8, 104.0, 87.8, 86.5, 84.2, 73.2, 70.3, 66.9, 63.8, 63.5, 29.1, 26.3, 25.8, 23.7; MALDI-HRMS *m/z* calcd 481.21967 $[M + Na]^+$, *m/z* found 481.21750. Anal. Calcd for $C_{26}H_{34}O_7 \cdot 0.1H_2O$: C, 67.84; H, 7.49. Found: C, 67.73; H, 7.51.

1,2-Di-*O*-Acetyl-3-*O*-benzyl-3-*C*-(3-benzyloxy)propyl-4-*O*-mesyl-4-*C*-mesyloxymethyl-D-*erythro*-pentofuranose (21). Furanose **20** (1.64 g, 3.60 mmol) was coevaporated with ice-cold anhydrous pyridine (10 mL) and dissolved in anhydrous pyridine (15 mL), and MsCl (1.3 mL, 7.7 mmol) was added dropwise. The solution was stirred for 1 h at rt followed by addition of ice-cold H_2O (20 mL). The resulting mixture was extracted using CH_2Cl_2 (2 × 20 mL) and evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (3 × 15 mL) and dissolved in TFA/H_2O (8:2 (v/v), 15 mL), and the resulting mixture was stirred for 3 h at rt. The mixture was evaporated to dryness under reduced pressure and coevaporated successively with toluene (4 × 15 mL) and pyridine (15 mL). The residue was dissolved in anhydrous pyridine (10 mL), and Ac_2O (2.0 mL, 21.2 mmol) was added. The resulting mixture was stirred for 17 h at rt, more Ac_2O (2.0 mL, 21.2 mmol) was added, and stirring was continued for additional 3 h. Ice (30 mL) was added, and after stirring for 5 min the mixture was evaporated to dryness. The residue was coevaporated with toluene (2 × 15 mL + 10 mL) and was purified by dry column vacuum chromatography (EtOAc/petroleum ether, 4:6−1:0 (v/v)) to give anomeric mixture **21** (2.05 g, 87%) as a white powder: R_f 0.37 (EtOAc/petroleum ether, 7:3 (v/v)); ^{13}C NMR ($CDCl_3$) 169.2, 138.4, 137.4, 128.7, 128.6, 128.0, 127.8, 127.8, 127.2, 127.0, 97.7, 87.3, 84.6, 79.3, 73.2, 69.6, 69.5, 68.5, 66.5, 65.7, 37.9, 37.6, 26.8, 26.8, 24.0, 21.1, 20.9; MALDI-HRMS *m/z* calcd 681.16460 $[M + Na]^+$, *m/z* found 681.16090.

1-(2-*O*-Acetyl-3-*O*-benzyl-3-*C*-(3-benzyloxy)propyl-5-*O*-mesyl-4-*C*-mesyloxymethyl-β-D-*erythro*-pentofuranosyl)-thymine (22). The anomeric mixture **21** (144.8 mg, 0.22 mmol) was coevaporated with anhydrous acetonitrile (5 mL) and dissolved in anhydrous acetonitrile (8 mL). Thymine (89.3 mg, 0.71 mmol) and *N*,*O*-bistrimethylsilyl acetamide (179 mg, 0.88 mmol) were added, and the stirred reaction mixture was heated under reflux until the solution turned clear (approximately after 1 h). The reaction mixture was cooled to 0 °C, trimethylsilyl triflate (146.6 mg, 0.66 mmol) was added dropwise, and the stirred reaction was heated under reflux for 23 h whereupon a satd aq solution of $NaHCO_3$ (50 mL) was added. Extraction was performed using CH_2Cl_2 (70 mL). The organic phase was washed with satd aq $NaHCO_3$ (50 mL) and evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (10 mL) and was purified by dry column vacuum chromatography (EtOAc/petroleum ether, 6:4−8:2 (v/v)) to give nucleoside **22** (148.2 mg, 93%) as a white solid material: R_f 0.31 (EtOAc/petroleum ether, 7:3 (v/v)); ^{1}H NMR ($CDCl_3$) 8.61 (br s, 1H, NH), 7.41−7.26 (m, 10H, Bn), 6.31 (d, 1H, J = 8.3 Hz, 1′-H), 5.51 (d, 1H, J = 8.3 Hz, 2′-H), 5.05 (d, 1H, J = 11.1 Hz, CH_2Ph), 4.65 (d, 1H, J = 11.1 Hz, CH_2Ph), 4.55−4.37 (m, 6H, 5′-H, 5″-H and CH_2Ph), 3.60−3.48 (m, 2H, C3′-CH_2CH_2CH_2OH), 2.92, 2.90 (2 × s, 2 × 3H, 2 × CH_3), 2.34−1.65 (m, 4H, C3′-CH_2CH_2CH_2-OH), 2.12, 1.94 (2 × s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) 169.6, 163.5, 150.9, 138.1, 137.7, 135.4, 128.8, 128.7, 128.6, 128.2, 128.0, 128.0, 127.4, 112.4, 86.0, 84.5, 84.2, 77.3, 73.5, 69.5, 68.6, 68.2, 67.1, 37.4, 37.4, 26.1, 24.2, 20.9, 12.3; MALDI-HRMS *m/z* calcd 747.18640 $[M + Na]^+$, *m/z* found 747.18940. Anal. Calcd for $C_{32}H_{40}N_2O_{13}S_2$: C, 53.03; H, 5.56; N, 3.87; S, 8.85. Found: C, 52.92; H, 5.65; N, 3.75; S, 8.73.

(1*S*,3*R*,4*R*,7*S*)-7-Benzyloxy-7-(3-benzyloxy)propyl-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]-heptane (23). Nucleoside **22** (2.04 g, 2.8 mmol) was dissolved in a mixture of 1,4-dioxane (15 mL), H_2O (15 mL), and an aqueous solution of 6 M KOH (30 mL, 0.18 mol). The stirred reaction mixture was heated under reflux for 45 h. To the reaction mixture was added brine (200 mL), and extraction was performed using dichloromethane (2 × 100 mL). The combined organic phase was evaporated to dryness under reduced pressure to give a residue that was coevaporated with toluene (2 × 25 mL) and then purified by dry column vacuum chromatography (MeOH/EtOAc, 0:1−1:9 (v/v)) to give the bicyclic nucleoside **23** (944.0 mg, 66%) as a white foam: R_f 0.45 (methanol/dichloromethane, 1:9 (v/v)); ^{1}H NMR ($CDCl_3$) 9.12 (br s, 1H, NH), 7.49 (d, 1H, J = 1.2 Hz, 6-H), 7.35−7.23 (m, 10H, H_{arom}), 5.49 (s, 1H, 1′-H) 5.10 (s, 1H, 2′-H), 4.64 (d, 1H, J = 10.4 Hz, CH_2Ph), 4.53 (d, 1H, J = 10.4 Hz, CH_2Ph), 4.43 (s, 2H, CH_2Ph), 4.38−3.95 (m, 4H, 5′-H and 5″-H), 3.40−3.25 (m, 2H, C3′-$CH_2CH_2CH_2$OH), 2.18 (1H, 5′-OH), 1.85 (s, 3H, CH_3), 1.79−1.56 (m, 4H, C3′-CH_2CH_2CH_2OH); ^{13}C NMR ($CDCl_3$) 164.0, 150.2, 138.2, 137.8, 134.7, 128.6, 128.5, 127.9, 127.8, 127.6, 127.6 (C_{arom}), 110.5, 91.0 (C-1′), 89.2, 84.0, 79.6 (C-2′), 74.6, 73.2, 69.7, 68.0, 59.1, 25.3, 23.9, 12.6; MALDI-HRMS *m/z* calcd 531.21017 $[M + Na]^+$, *m/z* found 531.20890. Anal. Calcd for $C_{28}H_{32}N_2O_7$: C, 65.20; H, 6.41; N, 5.43. Found: C, 65.32; H, 6.46; N, 5.35.

(1*R*,3*R*,4*R*,7*S*)-7-Benzyloxy-7-(3-benzyloxy)propyl-1-(4,4′-dimethoxytrityl)oxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (24). Nucleoside **23** (1.70 g, 3.3 mmol) was coevaporated with anhydrous pyridine (20 mL) and dissolved in anhydrous pyridine (30 mL) whereupon DMTCl (1.73 g, 5.1 mmol) was added. After the mixture was stirred for 19 h at rt, methanol (20 mL) was added and the resulting mixture was evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (150 mL) and washed with satd aq $NaHCO_3$ (3 × 100 mL). The organic phase was evaporated to dryness under reduced pressure to give a residue that was coevaporated with toluene (20 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 1:1−7:3 (v/v)) to give the bicyclic nucleoside **24** (2.44 g, 90%) as a clear oil: R_f 0.47 (methanol/dichloromethane, 1:19 (v/v)); ^{1}H NMR 8.96 (br s, 1H, NH), 7.51−7.14, 6.83−6.77 (2 × m, 24H, H_{arom}), 5.52 (s, 1H, 1′-H), 5.05 (s, 1H, 2′-H), 4.58−4.15 (m, 8H, 5′-H, 5″-H, CH_2Ph), 3.76 (s, 6H, 2 × CH_3), 3.56 (d, J = 10.7 Hz, 1H, CH_2Ph), 3.47 (d, J = 10.7 Hz, 1H, CH_2Ph), 3.22−3.15 (m, 2H, C3′-$CH_2CH_2CH_2$OH), 1.86 (s, 3H, CH_3), 1.72−1.34 (m, 4H, C3′-CH_2CH_2CH_2OH); ^{13}C NMR ($CDCl_3$) 163.9, 158.8, 158.7, 150.2, 144.6, 138.4, 138.1, 135.7, 135.6, 134.8, 130.3, 130.1, 128.5, 128.4, 128.2, 128.0, 127.7, 127.6, 127.6, 127.4, 127.1, 113.3, 110.4, 90.8 (C-1′), 89.0, 86.7, 84.0, 79.2 (C-2′), 74.9, 73.0, 69.7, 67.7, 60.0, 55.3, 25.2, 24.5, 12.7; MALDI-HRMS *m/z* calcd 833.34085 $[M + Na]^+$, *m/z* found 833.34220. Anal. Calcd for $C_{49}H_{50}N_2O_9 \cdot 0.5$EtOAc: C, 71.64; H, 6.37; N, 3.28. Found: C, 71.74; H, 6.37; N, 3.44.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityl)oxymethyl-7-hydroxy-7-(3-hydroxy)propyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (25). Nucleoside **24** (270 mg, 0.33 mmol) and a catalytic amount of 10% Pd/C was dissolved in methanol (20 mL), and $HCOONH_4$ (547 mg, 8.70 mmol) was added. The reaction mixture was heated under reflux for 6 h and subsequently filtered through silica gel, washing with absolute ethanol (6 × 25 mL) to furnish nucleoside **25** (156 mg, 74%) as a clear oil: R_f 0.12 (methanol/dichloromethane, 1:19 (v/v)); ^{1}H NMR ($CDCl_3$) 9.67 (br s, 1H, NH), 7.50−7.18, 6.87−6.80 (2 × m, 14H, H_{arom}), 5.45 (s, 1H, 1′-H), 4.78 (s, 1H, 2′-H), 4.31 (br s, 1H, OH), 4.19 (s, 2H, 5′-H), 3.79 (s, 6H, 2 × CH_3), 3.56−3.34 (m, 4H, 5″-H, C3′-$CH_2CH_2CH_2$OH), 3.10 (br s, 1H, OH), 1.91 (s, 3H, CH_3), 1.60−1.43, 1.34−1.23 (2 × m, 2 × 2H, C3′-CH_2CH_2CH_2OH); ^{13}C NMR ($CDCl_3$) 164.4, 158.8, 158.7, 150.6, 144.6, 135.8, 135.5, 134.6, 130.3, 130.1, 128.2, 128.0, 127.1, 113.4, 110.5, 90.0 (C-1′), 88.2, 86.6, 80.3, 80.0 (C-2′), 74.1, 62.4, 59.8, 55.4, 28.0, 27.3, 12.9; MALDI-HRMS *m/z* calcd 653.24695 $[M + Na]^+$, *m/z* found 653.24470. Anal. Calcd for $C_{35}H_{38}N_2O_9 \cdot 1.25H_2O$: C, 64.36; H, 6.25; N, 4.29. Found: C, 64.49; H, 6.60; N, 4.38.

(1*R*,3*R*,4*R*,7*S*)-7-(3-Acetoxypropyl)-1-(4,4′-dimethoxytrityl)oxymethyl-7-hydroxy-3-(thymin-1-yl)- 2,5-dioxabi-

cyclo[2.2.1]heptane (26). Nucleoside **25** (314 mg, 0.50 mmol) was coevaporated with anhydrous pyridine (15 mL) and dissolved in anhydrous pyridine (10 mL), and Ac_2O (0.1 mL, 1.1 mmol) was added. After the mixture was stirred for 26 h at rt, H_2O (1 mL) was added, and the resulting mixture was after 5 min evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (3 × 5 mL) and was purified by dry column vacuum chromatography (EtOAc/petroleum ether, 1:1−7:3 (v/v)) yielding nucleoside **26** as a white foam (302 mg, 90%): R_f 0.64 (methanol/dichloromethane, 1:9 (v/v)); ^{1}H NMR ($CDCl_3$) 7.51−7.22, 6.88−6.82 (2 × m, 14H, H_{arom}), 5.45 (s, 1H, 1′-H), 4.72 (s, 1H, 2′-H), 4.20−4.09, 3.96−3.82 (2 × m, 2 × 2H, 5′-H, 5″-H), 3.80, 3.80 (2 × s, 2 × 3H, 2 × CH_3), 3.54 (d, 1H, J = 10.9 Hz, C3′-$CH_2CH_2CH_2OH$), 3.36 (d, 1H, J = 10.9 Hz, C3′-$CH_2CH_2CH_2OH$), 1.96 (s, 3H, CH_3), 1.50−1.42 (m, 7H, CH_3, C3-$CH_2CH_2CH_2OH$); ^{13}C NMR ($CDCl_3$) 171.1, 163.7, 158.8, 158.8, 150.2, 144.5, 135.6, 135.3, 134.4, 130.2, 130.1, 128.2, 128.1, 127.2, 113.4, 110.5, 89.7 (C-1′), 87.9, 86.8, 80.2 (C-2′), 80.0, 73.7, 64.4, 59.6, 55.4, 26.2, 24.4, 21.0, 12.9; MALDI-HRMS m/z calcd 695.25751 $[M + Na]^+$, m/z found 695.25750.

(1*R*,3*R*,4*R*,7*S*)-7-(3-Acetoxypropyl)-7-(2-cyanoethoxy)-(*N*,*N*-(diisopropyl)amino)phosphinoxy)-1-(4,4′-dimethoxytrityl)oxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (27). Nucleoside **26** (144 mg, 0.20 mmol) was dissolved in anhydrous CH_2Cl_2 (5 mL) and *N*,*N*-(diisopropyl)-ethylamine (0.15 mL, 0.8 mmol), and 2-cyanoethyl *N*,*N*-(diisopropyl)phosphoramidochloridite (0.10 mL, 0.4 mmol) was added. The reaction mixture was stirred for 26 h at rt, whereupon methanol (2.5 mL) was added followed by dichloromethane (5 mL). The resulting mixture was washed successively with satd aq $NaHCO_3$ (3 × 10 mL), H_2O (10 mL), and brine (10 mL). The combined aqueous phase was extracted with dichloromethane (10 mL), and the combined organic phase was evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (10 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 3:2−7:3 (v/v)) to give phosphoramidite **27** (120 mg, 64%) as a clear oil: R_f 0.72 (methanol/dichloromethane, 1:9 (v/v)); ^{31}P NMR ($CDCl_3$) 146.2, 145.8; MALDI-HRMS m/z calcd 895.36537 $[M + Na]^+$, m/z found 895.36160.

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-3-*C*-(3-hydroxy)-propyl-α-D-allofuranose (28). To a stirred solution of furanose **6** (285 mg, 0.73 mmol) in anhydrous THF (5 mL) was added dropwise a 2 M solution of BH_3·Me_2S in diethyl ether (1.1 mL, 2.2 mmol) at 0 °C. After being stirred for 4 h at rt, the reaction mixture was cooled to 0 °C, and a mixture of 2 M aq NaOH (1.4 mL, 2.8 mmol) and 35% aq H_2O_2 (0.3 mL) was added dropwise. The reaction mixture was stirred for 67 h at rt followed by extraction using EtOAc (25 mL). The organic phase was washed with brine (2 × 25 mL) and the combined aqueous phase extracted with EtOAc (25 mL). The combined organic phase was evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (10 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 1:1−3:2 (v/v)) to give furanose **28** (137 mg, 46%) as a white powder: R_f 0.22 (EtOAc/petroleum ether, 7:3 (v/v)); ^{1}H NMR ($CDCl_3$) 7.40−7.22 (m, 5H, Bn), 5.65 (d, J = 3.7, 1H, 1-H), 4.87 (d, J = 11.2, 1H, CH_2Ph), 4.73 (d, J = 11.2, 1H, CH_2Ph), 4.49 (d, J = 3.7, 1H, 2-H), 4.30−3.92 (m, 4H, 4-H, 5-H, 6-H), 3.65 (*t*, J = 5.9, 2H, C3−$CH_2CH_2CH_2OH$), 1.99−1.63 (m, 4H, C3−$CH_2CH_2CH_2OH$), 1.60, 1.39, 1.35, 1.35 (4 × s, 4 × 3H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) 139.3, 128.2, 127.3, 112.9, 109.8, 103.3 (C-1), 84.0, 83.8 (C-2), 81.1, 73.1, 68.5, 66.9, 63.1, 27.5, 27.2, 26.7, 26.4, 25.5; MALDI-HRMS m/z calcd 431.20402 $[M + Na]^+$, m/z found 431.20260. Anal. Calcd for $C_{22}H_{32}O_7$·$0.2H_2O$: C, 63.84; H, 7.92. Found: C, 63.98; H, 7.90.

3-*O*-Benzyl-1,2:5,6-di-*O*-isopropylidene-3-*C*-(3-mesyloxy)-propyl-α-D-allofuranose (29). Compound **28** (3.77 g, 9.2 mmol) was coevaporated with anhydrous pyridine (20 mL) and dissolved in ice-cold anhydrous pyridine (40 mL), and mesyl chloride (1.0 mL, 12.9 mmol) was added dropwise. The solution was stirred for 3 h at rt whereupon ice-cold H_2O (200 mL) was added. The resulting mixture was extracted using CH_2Cl_2 (4 × 100 mL), and the combined organic phase was washed with satd aq $NaHCO_3$ (200 mL) and evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (2 × 20 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 1:3−1:0 (v/v)) yielding furanoside **29** (3.54 g, 79%) as a clear oil: R_f 0.55 (EtOAc/petroleum ether, 7:3 (v/v)); ^{1}H NMR ($CDCl_3$) 7.34−7.16 (m, 5H, Bn), 5.59 (d, 1H, J = 3.7 Hz, 1-H), 4.77 (d, J = 11.1 Hz, 1H, CH_2Ph), 4.68 (d, J = 11.1 Hz, 1H, CH_2Ph), 4.38 (d, J = 3.7 Hz, 1H, 2-H), 4.20−3.86 (m, 6H**,** 4-H, 5-H, 6-H, C3-$CH_2CH_2CH_2OH$), 2.89 (s, 3H, CH_3), 2.10−1.56 (m, 4H, C3-$CH_2CH_2CH_2OH$), 1.53, 1.33, 1.29, 1.28 (4 × s, 4 × 3H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) 139.1, 128.3, 127.3, 127.2, 113.0, 109.9, 103.2 (C-1), 84.0, 83.9, 80.6, 73.1, 70.4, 68.7, 66.9, 37.5, 27.2, 27.1, 26.7, 26.7, 25.5, 23.4; MALDI-HRMS m/z calcd 509.18157 $[M + Na]^+$, m/z found 509.18310.

3-*C*-(3-Azidopropyl)-3-*O*-benzyl-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (30). To a stirred solution of furanose **29** (361 mg, 0.74 mmol) in anhydrous DMF (4 mL) was added NaN_3 (121.0 mg, 1.9 mmol). The reaction mixture was heated to 100 °C and stirred for 24 h. H_2O (25 mL) was added, and extraction was performed using EtOAc (2 × 25 mL). The combined organic phase was washed successively with brine (20 mL), satd aq $NaHCO_3$ (20 mL), and H_2O (20 mL) and evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (15 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 1:9−12−88 (v/v)) affording furanose **30** (271 mg, 84%) as a clear oil: R_f 0.71 (EtOAc/petroleum ether, 7:3 (v/v)); ^{1}H NMR ($CDCl_3$) 7.40−7.22 (m, 5H, Bn), 5.65 (d, 1H, J = 3.6 Hz, 1-H), 4.83 (d, J = 11.3 Hz, 1H, CH_2Ph), 4.73 (d, J = 11.3 Hz, 1H, CH_2Ph), 4.45 (d, J = 3.6 Hz, 1H, 2-H), 4.21−3.91 (m, 4H, 4-H, 5-H, 6-H), 3.37−3.28 (m, 2H, C3-$CH_2CH_2CH_2OH$), 1.95−1.62 (m, 4H, C3-$CH_2CH_2CH_2OH$), 1.60, 1.39, 1.36, 1.35 (4 × s, 4 × 3H, 4 × CH_3); ^{13}C NMR ($CDCl_3$) 139.2, 128.2, 127.3, 127.1, 113.0, 109.8, 103.3 (C-1), 84.0 (C-2), 81.1, 73.2, 68.7, 66.9, 52.0, 28.2, 27.1, 26.7, 25.5, 23.0. MALDI-HRMS m/z calcd 456.21050 $[M + Na]^+$, m/z found 456.21090; IR 2098 cm^{-1} (−N_3). Anal. Calcd for $C_{22}H_{31}N_3O_6$: C, 60.95; H, 7.21; N, 9.69. Found: C, 61.18; H, 7.22; N, 9.82.

3-*C*-(3-Azido)propyl-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-allofuranose (31). Compound **30** (208 mg, 0.48 mmol) was dissolved in a mixture of AcOH and H_2O (8:2 (v/v), 5 mL) and stirred for 41 h at rt. The reaction mixture was evaporated and the residue coevaporated with toluene (2 × 10 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 1:1−3:2 (v/v)) to give furanoside **31** (155 mg, 82%) as a clear oil: R_f 0.44 (EtOAc/petroleum ether, 7:3 (v/v); ^{1}H NMR ($CDCl_3$) 7.38−7.26 (m, 5H, Bn), 5.70 (d, J = 3.6 Hz, 1H, 1-H), 4.74 (d, J = 10.5 Hz, 1H, CH_2Ph), 4.64 (d, J = 10.5 Hz 1H, CH_2Ph), 4.46 (d, J = 3.6 Hz, 1H, 2-H), 4.11 (d, J = 9.0 Hz, 1H, 4-H), 3.85−3.63 (m, 3H, 5-H, 6-H), 3.39−3.34 (m, 2H, C3-$CH_2CH_2CH_2OH$), 2.73 (br s, 1H, OH), 2.24 (br s, 1H, OH), 1.96−1.74 (m, 4H, C3-$CH_2CH_2CH_2OH$), 1.61, 1.36 (2 × s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) 137.9, 128.5, 127.9, 127.7, 113.3, 104.0 (C-1), 84.9, 82.7 (C-2), 78.8, 69.8, 67.7, 64.8, 52.0, 28.4, 26.9, 26.7, 23.3; MALDI-HRMS m/z calcd 416.17920 $[M + Na]^+$, m/z found 416.18080; IR 2098 cm^{-1} (−N_3). Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 57.70; H, 6.93; N, 10.44.

3-*C*-(3-Azidopropyl)-3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-*erythro*-pentofuranose (32). To a stirred ice-cold solution of $NaIO_4$ (2.16 g, 10.1 mmol) in a mixture of H_2O and 1,4-dioxane (2:1 (v/v), 90 mL) was dropwise added furanose **31** (2.21 g, 5.6 mmol) dissolved in THF (30 mL). Ethylene glycol (2.5 mL) was added after 5 h, the mixture was filtered, and the filtering funnel was washed with EtOAc (2 × 50 mL). The filtrate was extracted with EtOAc (100 mL), and the combined organic phase was evaporated to dryness under reduced pressure. The residue was dissolved in 1,4-

dioxane (40 mL), and formaldehyde (2.0 mL, 37% aqueous solution (w/w), 50.4 mmol) and 1 M aq NaOH (40 mL, 40 mmol) were added. After the mixture was stirred for 20 h at rt, a 17.45 N aqueous solution of formic acid was added to neutralize the mixture. H_2O (50 mL) was added, and the resulting mixture was extracted with EtOAc (3 × 100 mL). The combined organic phase was evaporated to dryness, and the residue was coevaporated with toluene (2 × 20 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 2:3−3:2 (v/v)) to give furanose **32** (1.96 g, 89%) as a white foam: R_f 0.35 (EtOAc/petroleum ether, 7:3 (v/v)); ^{1}H NMR ($CDCl_3$) 7.39−7.26 (m, 5H, Bn), 5.79 (d, J = 4.3 Hz, 1H, 1-H), 4.71 (d, J = 10.6 Hz, 1H, CH_2Ph), 4.57 (d, J = 10.6 Hz, 1H, CH_2Ph), 4.50 (d, J = 4.3 Hz, 1H, 2-H), 4.13 (d, J = 12.0 Hz, 1H, 5-H), 4.06 (d, J = 12.0 Hz, 1H, 5-H), 3.85−3.80 (m, 2H, 5-H′), 3.45−3.31 (m, 2H, C3-$CH_2CH_2CH_2$OH), 2.56 (br s, 1H, OH), 2.47 (br s, 1H, OH), 2.08−1.74 (m, 4H, C3-$CH_2CH_2CH_2$OH), 1.66, 1.34 (2 × s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) 138.0, 128.6, 128.0, 127.6, 113.0, 103.9, 87.7, 86.3, 84.4, 67.0, 63.7, 63.5, 52.0, 29.8, 26.3, 25.8, 23.0; MALDI-HRMS m/z calcd 416.17920 $[M + Na]^+$, m/z found 416.17900; IR 2098 cm^{-1} (−N_3). Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 58.00; H, 6.92; N, 10.68. Found: C, 57.76; H, 6.93; N, 10.20.

3-*C*-(3-Azidopropyl)-1,2-di-*O*-acetyl-3-*O*-benzyl-5-*O*-mesyl-4-*C*-mesyloxymethyl-D-*erythro*-pentofuranose (33). Compound **32** (1.95 g, 5.0 mmol) was coevaporated with anhydrous pyridine (20 mL) and dissolved in ice-cold anhydrous pyridine (30 mL), and MsCl (0.96 mL, 12.4 mmol) was added dropwise under stirring. The solution was stirred for 4.5 h at rt followed by addition of satd aq $NaHCO_3$ (50 mL) and brine (100 mL). The resulting mixture was extracted with CH_2Cl_2 (3 × 50 mL), and the combined organic phase was evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (2 × 20 mL) and then dissolved in a mixture of TFA and H_2O (8:2 (v/v), 25 mL). After stirring for 3.5 h at rt, the mixture was evaporated to dryness under reduced pressure, and the residue was coevaporated successively with toluene (2 × 30 mL) and anhydrous pyridine (20 mL). The resulting residue was dissolved in anhydrous pyridine (30 mL) at rt under stirring, and Ac_2O (4.8 mL, 50.8 mmol) and DMAP (79 mg, 0.6 mmol) were added. The reaction mixture was stirred for 15 h at rt followed by addition of ice (30 mL) and satd aq $NaHCO_3$ (50 mL). Extraction was performed using CH_2Cl_2 (3 × 100 mL), and the combined organic phase was first evaporated to dryness under reduced pressure and then coevaporated with toluene (2 × 20 mL). The residue was purified by dry column vacuum chromatography (EtOAc/petroleum ether, 2:3 (v/v)) to give anomeric mixture **33** (1.98 g, 67%) as a white foam: R_f 0.55 (EtOAc/petroleum ether, 7:3 (v/v)); ^{13}C NMR ($CDCl_3$) 169.3, 169.1, 137.1, 128.8, 128.1, 127.3, 127.0, 97.6, 87.3, 84.5, 79.1, 68.4, 66.7, 65.6, 51.3, 38.0, 37.7, 37.6, 27.2, 23.2, 21.1, 20.8; MALDI-HRMS m/z calcd 616.12413 $[M + Na]^+$, m/z found 616.12170; IR 2099 cm^{-1} (−N_3).

1-(2-*O*-Acetyl-3-*C*-(3-azidopropyl)-3-*O*-benzyl-5-*O*-mesyl-4-*C*-mesyloxymethyl-β-D-*erythro*-pentofuranosyl)thymine (34). To a stirred solution of the anomeric mixture **33** (1.01 mg, 1.7 mmol) in anhydrous acetonitrile (20 mL) were added thymine (644 mg, 5.1 mmol) and *N*,*O*-bistrimethylsilyl acetamide (1.68 mL, 6.8 mmol). The mixture was stirred with heating under reflux until the solution became clear (45 min) and was then cooled to 0 °C followed by addition of trimethylsilyl triflate (0.92 mL, 5.1 mmol). The mixture was heated under reflux for 43 h whereupon satd aq $NaHCO_3$ (50 mL) was added. Extraction was performed using CH_2Cl_2 (3 × 50 mL), the combined organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene (25 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 2:3−3:2 (v/v)) yielding nucleoside **34** (841 mg, 75%) as a white foam: R_f 0.25 (EtOAc/petroleum ether, 7:3 (v/v); ^{1}H NMR ($CDCl_3$) 9.11 (s, 1H, NH), 7.43−7.26 (m, 6H, Bn), 6.20 (d, J = 8.0 Hz, 1H, 1-H′), 5.61 (d, J = 8.0 Hz, 1H, 2-H′), 5.01−4.41 (m, 6H, 5-H′, 5-H″, CH_2Ph), 3.51−3.37 (m, 2H, C3′-$CH_2CH_2CH_2$OH), 3.15, 2.94 (2 × s, 2 × 3H, 2 × CH_3), 2.33−1.67 (m, 4H, C3′-$CH_2CH_2CH_2$OH), 2.14, 1.94 (2 × s, 2 × 3H, 2 × CH_3); ^{13}C NMR ($CDCl_3$) 169.6, 163.6, 150.9, 137.4, 135.8, 128.9, 128.3, 127.4, 112.3, 85.9 (C-1′), 85.4, 83.9, 76.9 (C-2′), 68.1, 67.6, 67.1, 51.2, 37.8, 37.5, 26.3, 23.3, 20.9, 12.4; MALDI-HRMS m/z calcd 682.14593 $[M + Na]^+$, m/z found 682.14400; IR 2101 cm^{-1} (−N_3). Anal. Calcd for $C_{25}H_{33}N_5O_{12}S_2$: C, 45.52; H, 5.04; N, 10.62. Found: C, 45.70; H, 5.10; N, 10.49.

(1*S*,3*R*,4*R*,7*S*)-7-(3-Azidopropyl)-7-benzyloxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]-heptane (35). Nucleoside **34** (825 mg, 1.3 mmol) was dissolved in a mixture of 1,4-dioxane (6 mL), H_2O (6 mL), and aq 6 M KOH (12 mL, 72 mmol). The reaction mixture was stirred under reflux for 41 h followed by the addition of brine (100 mL). Extraction was performed using EtOAc (3 × 80 mL), the combined organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene (20 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 3:2−7:3 (v/v)) to give the bicyclic nucleoside **35** (350 mg, 63%) as a white foam: R_f 0.69 (methanol/dichloromethane, 1:1 (v/v)); ^{1}H NMR ($CDCl_3$) 9.28 (br s, 1H, NH), 7.45 (s, 1H, 6-H), 7.30−7.19 (m, 5H, Bn), 5.43 (s, 1H, 1-H′), 5.05 (s, 1H, 2-H′), 4.62−3.89 (m, 6H, 5-H′, 5-H″, CH_2PH), 3.34−3.02 (m, 2H, C3′-$CH_2CH_2CH_2$OH), 2.20 (br s, 1H, OH), 1.90 (s, 3H, CH_3), 1.71−1.48 (m, 4H, C3′-$CH_2CH_2CH_2$OH); ^{13}C NMR ($CDCl_3$) 164.1, 150.3, 137.5 (C-6), 134.5, 128.6, 128.6, 128.0, 127.7, 127.6, 110.9, 91.0, 89.2 (C-1′), 83.8, 79.6 (C-2′), 74.5, 68.1, 59.0, 51.7, 24.5, 24.4, 12.7; MALDI-HRMS m/z calcd 466.16970 $[M + Na]^+$, m/z found 466.1700; IR 2099 cm^{-1} (−N_3).

(1*S*,3*R*,4*R*,7*S*)-7-(3-(Fluoren-9-ylmethoxycarbonyl)aminopropyl)-7-hydroxy-1-hydroxymethyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (36). Nucleoside **35** (293 mg, 0.7 mmol) was dissolved in methanol at rt under stirring (15 mL), and $Pd(OH)_2$/C (105 mg, 0.1 mmol) was added. The reaction flask was evacuated and filled with hydrogen gas several times. The reaction mixture was stirred vigorously under an atmosphere of hydrogen gas for 42 h at rt and was then filtered through filter paper that was washed with methanol (3 × 25 mL). The combined filtrate was evaporated to dryness under reduced pressure, and the obtained residue was coevaporated with anhydrous pyridine (5 mL) and then dissolved in anhydrous pyridine (10 mL). Fluoren-9-ylmethoxycarbonyl chloride (175 mg, 0.7 mmol) was added, and the reaction mixture was stirred for 19 h at rt followed by addition of satd aq $NaHCO_3$ (70 mL). Extraction was performed using EtOAc (100 mL), the organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene (25 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 3:2 (v/v); methanol/EtOAc, 3:50 (v/v)) affording nucleoside **36** (158 mg, 51%) as a white powder: R_f 0.10 (EtOAc); ^{1}H NMR (DMSO-d_6) 7.76−7.18 (m, 7H, Bn), 6.41−6.14 (m, 2H, Bn), 5.08 (s, 1H, 1-H′), 4.20 (s, 1H, 2-H′), 3.94−3.56 (m, 8H, 5-H′, 5-H″, C3′-$CH_2CH_2CH_2$OH and $CHCH_2$), 2.60, 2.37 (2 × br s, 2 × 1H, 2 × OH), 1.68 (s, 3H, CH_3), 1.44−0.68 (m, 4H, C3′-$CH_2CH_2CH_2$OH), 1.04 (t, J = 7.1 Hz, 1H, CH); ^{13}C NMR ($CDCl_3$) 162.8, 155.0, 149.3, 143.1, 142.9, 139.7, 133.4, 126.6, 126.1, 124.2, 119.1, 107.5, 89.6, 86.7, 78.8 (C-2′), 77.8, 72.0, 64.4, 58.8, 55.9, 45.7, 39.8, 26.4, 24.1, 13.1, 11.3; MALDI-HRMS m/z calcd 572.20033 $[M + Na]^+$, m/z found 572.19960.

(1*R*,3*R*,4*R*,7*S*)-1-(4,4′-Dimethoxytrityl)oxymethyl-7-(3-(fluoren-9-yl-methoxycarbonyl)aminopropyl)-7-hydroxy-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (37). Nucleoside **36** (158 mg, 0.3 mmol) was coevaporated with anhydrous pyridine (5 mL) and then dissolved in anhydrous pyridine (10 mL). DMTCl (153 mg, 0.5 mmol) was added, and the reaction mixture was stirred for 2 h at rt. Methanol (1.5 mL) was added, and the resulting mixture was extracted with EtOAc (30 mL). The organic phase was washed with satd aq $NaHCO_3$ (2 × 20 mL), and the combined aqueous phase was

extracted with CH_2Cl_2 (20 mL). The combined organic phase was evaporated to dryness under reduced pressure, and the residue was coevaporated with toluene (15 mL) and purified by dry column vacuum chromatography (EtOAc/petroleum ether, 1:1−3:2 (v/v)) to give nucleoside **37** (166 mg, 68%, rotamers observed in NMR) as a white powder: R_f 0.69 (EtOAc); ^{1}H NMR ($CDCl_3$) 9.33, 9.24 (2 × br s, 1H, NH), 7.71−6.82 (m, 22H, H_{arom}), 5.42, 5.34 (2 × s, 1H, 1-H′), 4.69 (s, 1H, 2-H′), 4.36−3.92 (m, 6H, 5-H′, 5-H″, C3′-$CH_2CH_2CH_2OH$), 3.79, 3.76 (2 × s, 2 × 3H, 2 × CH_3), 3.51 (d, 1H, J = 10.8 Hz, CH_2-CH), 3.35 (d, 1H, J = 10.8 Hz, CH_2CH), 3.25 (br s, 1H, OH), 1.92, 1.89 (2 × s, 3H, CH_3), 1.81−1.11 (m, 5H, C3′-CH_2CH_2-CH_2OH, CH); ^{13}C NMR ($CDCl_3$) 163.8, 163.8, 158.8, 158.8, 158.8, 156.7, 150.3, 147.5, 144.6, 144.2, 144.1, 144.0, 141.4, 139.6, 135.6, 135.4, 134.2, 134.2, 130.2, 130.1, 129.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.2, 127.2, 125.2, 120.0, 113.4, 113.3, 110.7, 110.6, 89.9 (C-1′), 89.9, 88.1, 88.0, 86.8, 81.6, 80.4, 80.3 (C-2′), 80.0, 73.8, 73.3, 66.9, 59.5, 58.6, 55.4, 55.4, 47.3, 41.1, 29.8, 26.6, 26.4, 25.8, 12.9, 12.8; MALDI-HRMS *m*/*z* calcd 874.33101 $[M + Na]^+$, *m*/*z* found 874.33480.

(1*R*,3*R*,4*R*,7*S*)-7-(2-Cyanoethoxy)(*N*,*N*-(diisopropylamino)phosphinoxy)-1-(4,4′-dimethoxytrityl)oxymethyl-7-(3-(fluoren-9-yl-methoxycarbonyl)amino)propyl-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (38). Nucleoside **37** (140 mg, 0.2 mmol) was coevaporated with anhydrous acetonitrile (2 mL) and then dissolved in anhydrous CH_2Cl_2 (2 mL). *N*,*N*-(Diisopropyl)ethylamine (0.12 mL, 0.8 mmol) and 2-cyanoethyl *N*,*N*-(diisopropyl)phosphoramidochloridite (0.06 mL, 0.3 mmol) were added, and the resulting mixture was stirred for 24 h at rt. CH_2Cl_2 (20 mL) was added, and the resulting mixture was washed with satd aq $NaHCO_3$ (25 mL). The organic phase was evaporated to dryness under reduced pressure, and the resulting mixture was coevaporated with acetonitrile (5 mL) and purified by dry column vacuum chromatography using EtOAc/petroleum ether (9:11−1:1 (v/v)) to furnish phosphoramidite **38** (151 mg, 87%) as a yellowish oil: R_f 0.52 (methanol/dichloromethane, 1:9 (v/v)); ^{31}P NMR ($CDCl_3$) 145.8, 145.5; MALDI-HRMS *m*/*z* calcd 1074.43886 $[M + Na]^+$, *m*/*z* 1074.43650.

Synthesis, Deprotection, and Purification of LNA ONs. LNA oligomers **LNA-1**, **LNA-2**, and **LNA-3** were prepared by the phosphoramidite approach as described earlier on an automated DNA synthesizer on CPG solid support (**LNA-1** and **LNA-2**) or polystyrene solid support (**LNA-3**; and also **LNA-4** by on-column conjugation). The stepwise coupling efficiencies for LNA phosphoramidites **27** and **38** were ∼85% (15 min coupling time using pyridinium chloride as catalyst for **27**; 30 min coupling time using 1*H*-tetrazole as catalyst for **38**) and for unmodified deoxynucleoside phosphoramidites (with 2 min standard coupling time) >99% using 1*H*-tetrazole as activator. After standard deprotection and cleavage from the solid support by 32% aqueous ammonia, **LNA-1** and **LNA-2** were purified by DMT-on reversed-phase chromatography on disposable purification cartridges, which included detritylation. **LNA-3** was purified by HPLC using an X-Terra MS C_{18} column (7.8 × 300 mm) and 254 nm UV detection; buffer A: MeCN:0.1 M NH_4HCO_3 (5:95 v/v); buffer B: MeCN: 0.1 M NH_4HCO_3 (75:25 v/v). Gradients: 5 min 100% A, linear gradient to 70% B in 30 min, linear gradient to 100% B in 2 min (flow 1.5 mL/min), 8 min with 100% B (flow 1.5 mL/min), linear gradient to 100% A in 1 min and 14 min with 100%. The flow rate was 1.0 mL/min. The composition of the synthesized ONs was confirmed by MALDI-MS analysis and the purity by capillary gel electrophoresis/analytical RP-HPLC. MALDI-MS: **LNA-1** $[M - H]^-$ 3008.8, calcd 3012.1; **LNA-2** $[M - H]^-$ 3009.9, calcd 3008.5; **LNA-3** $[M - H]^-$ 2837.9, calcd 2838.7. ON **LNA-3** (0.3 μmol), bound to a polystyrene solid support following incorporation of the last nucleotide, was suspended in a solution of 20% piperidine in anhydrous DMF (1.0 mL). After the reaction mixture was vortexed for 20 min at rt, the supernatant was removed and the support was washed with anhydrous CH_3CN (4 × 1.0 mL). To the resulting dry support were then added HBTU (5.90 mg, 15.6 μmol), Fmoc-glycine (4.68 mg, 18.8 μmol), DIPEA (0.01 mL), and anhydrous DMF (1.0 mL). After the resulting mixture was vortexed for 1 h, the supernatant was removed and the solid support washed with first DMF (2 × 1.0 mL) and then MeOH (2 × 1.0 mL). The solid support was subsequently suspended in satd aq ammonia (1.0 mL) and left for 19 h at 55 °C whereupon the liquid phase was removed and evaporated to dryness. The resulting ON was purified by reversed-phase HPLC (as described above) and evaporated to dryness. The residue was treated with 80% aqueous AcOH (100 μL, w/w) for 20 min, H_2O (100 μL) and 3 M aqueous CH_3COONa (50 μL) were added, the solution was transferred to an Eppendorf tube, and absolute EtOH (500 μL) was added. The resulting mixture was left at −20 °C for 22 h and centrifuged at 8000 rpm for 20 min. The supernatant was removed and the precipitated **LNA-4** isolated. MALDI-MS: **LNA-4** $[M - H]^-$ 2894.5, calcd 2896.0.

Thermal Denaturation Studies. The thermal denaturation studies were performed in a low salt buffer (10 mM sodium phosphate and 0.1 mM EDTA, pH 7.0) or in a medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride and 0.1 mM EDTA, pH 7.0). Concentrations of 1.0 μM of the two complementary ON strands were used assuming identical extinction coefficients for modified and unmodified ONs. 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.

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Supporting Information Available: General methods. Copies of ^{13}C NMR spectra of compounds **26**, **29**, **32**, **33**, and **35**−**37**. This material is available free of charge via the Internet at http://pubs.acs.org.

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