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Synthesis and RNA-selective hybridization of α-L-*ribo*- and β-D-*lyxo*-configured oligonucleotides

Gilles Gaubert,[a] B. Ravindra Babu,[a] Stefan Vogel,[a] Torsten Bryld,[a] Birte Vester[b] and Jesper Wengel[a,*]

[a]*Department of Chemistry, Nucleic Acid Center, University of Southern Denmark, DK-5230 Odense M, Denmark*
[b]*Department of Biochemistry and Molecular Biology, University of Southern Denmark, DK-5230 Odense M, Denmark*

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Abstract—Three α-L-ribofuranosyl analogues of RNA nucleotides (α-L-RNA analogues) have been synthesized and incorporated into oligonucleotides using the phosphoramide approach on an automated DNA synthesizer. The 4′-*C*-hydroxymethyl-α-L-ribofuranosyl thymine monomer was furthermore synthesized. Relative to the unmodified duplexes, incorporation of a single α-L-RNA monomer into a DNA strand leads to reduced thermal stability of duplexes with DNA complements but unchanged thermal stability of duplexes with RNA complements, whereas incorporation of more than one α-L-RNA monomer lead to moderately decreased thermal stability also of duplexes with RNA complements. Efficient hybridization with an RNA complement and no melting transition with a DNA complement were observed with stereoregular chimeric oligonucleotides composed of a mixture of α-L-RNA and affinity enhancing α-L-LNA monomers (α-L-*ribo*-configured locked nucleic acid). Furthermore, duplexes formed between oligodeoxynucleotides containing an α-L-RNA monomer and complementary RNA were good substrates for *Escherichia coli* RNase H. RNA-selective hybridization was also achieved by the incorporation of 1-(4-*C*-hydroxymethyl-β-D-lyxofuranosyl)thymine monomers into a DNA strand, whereas stable duplexes were formed with both complementary DNA and RNA when these monomers were incorporated into an RNA strand.

1. Introduction

The utilization of modified oligonucleotides (ONs) in the antisense approach requires the formation of duplexes with mRNA in order to specifically inhibit their translation into proteins involved in various pathologic disorders. Essential properties of successful antisense oligonucleotides (AON) are good aqueous solubility, resistance against enzymatic degradation, high binding affinity and specificity for the target RNA strand.[1] It is furthermore desirable if they have the ability to recruit the endogenous enzyme RNase H. An AON basically has two possible modes of action, which both involve hybridization to the RNA target. One is steric blocking of the mRNA, and the other is recognition of the RNA·AON duplex as a substrate for the enzyme RNase H, which subsequently cleaves the RNA strand of the duplex. In the latter scenario, one AON is able to pacify multiple mRNA strands. A high binding affinity towards RNA is crucial, especially for the steric blocking approach. Conformational restriction of the single-stranded AON has the potential to favour duplex formation entropically by diminishing the loss of conformational freedom upon duplex formation. α-L-LNA (α-L-*ribo*-configured locked nucleic acid),[2,3] containing a 2′-*O*,4′-*C*-methylene linked furanose ring,[†] with three out of four chirality centers inverted relative to RNA, forms duplexes with complementary RNA and DNA with highly increased thermal stability and generally improved selectivity. α-L-LNA can be most adequately described as a DNA mimic as NMR spectroscopic studies of α-L-LNA·RNA duplexes and molecular dynamics simulation of fully modified α-L-LNA·RNA duplexes have shown the overall duplex geometry to be very similar to the corresponding unmodified DNA·RNA hybrid.[3] Fully modified and mix-meric α-L-LNA (consisting

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* Corresponding author. Tel.: +45 65502510; fax: +45 66158780; e-mail: jwe@chem.sdu.dk

[†] α-L-LNA (α-L-*ribo* configured diastereoisomer of LNA; defined as an oligonucleotide containing one or more 2′-*O*,4′-*C*-methylene-α-L-ribofuranosyl nucleotide monomers) has shown appealing hybridization properties despite its unnatural configuration. The furanose conformation of an α-L-LNA monomer is of N-type (C3′-*endo*, ^3E). For further information about the conformations of the nucleotides, see *Eur. J. Biochem.* **1983**, *131*, 9 (abbreviations and symbols for the description of conformations of polynucleotide chains, IUPAC-IUB Joint Commission on Biochemical Nomenclature). A similar, but more flexible, furanose conformation is likely for an α-L-RNA monomer.[5]

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of a mixture of α-L-LNA and unmodified DNA nucleotides) supported in vitro *Escherichia coli* RNase H-mediated cleavage of the RNA target, albeit at a very reduced rate and at high enzyme concentration.[3c] RNase H has been reported to bind to the minor groove of substrate RNA·DNA heteroduplexes adopting a duplex form intermediate between the A- and B-form, with a minor groove width also intermediate between that of the A- and B-forms.[4] The furanose conformations of the nucleotides in the RNA strand are of the N-type, whereas hybridization of a DNA strand to the RNA strand causes the furanose conformation of the DNA strand to change from the typical *S*-type (C2′-*endo*) into *E*-type conformations (O4′-*endo* range).[4] Thus, the activation of RNase H proposedly requires AONs with furanose rings able to adopt *E*-type (O4′-*endo*), or perhaps *S*-type (C2′-*endo*), conformations. The locked furanose conformations of α-L-LNA might therefore explain the limited ability of α-L-LNA·RNA duplexes to act as substrates for RNase H (high enzyme concentration and extended reaction time) despite the global DNA-mimicking nature of α-L-LNA in α-L-LNA:RNA duplexes.[3c]

α-L-RNA (α-L-*ribo*-configured RNA) has structural resemblance to α-L-LNA. The furanose conformation of an α-L-LNA monomer is of the *N*-type (C3′-*endo*, ^{3}E),[†] and a similar furanose conformation, although more flexible, is likely to be preferred for an α-L-RNA monomer as indicated by calculations.[5] We had previously in a preliminary form reported the synthesis and binding properties of the α-L-RNA monomer bearing a thymine unit as nucleobase ($^{\alpha L}$**T**, Fig. 1).[6] The α-L-RNA thymine monomer when incorporated into an ON impairs a higher tendency towards hybridization with an RNA complement than with a DNA complement. A single incorporation of an α-L-RNA nucleotide in a 9-mer mixed-base sequence (**ON3**) leads to unchanged thermal stability towards RNA and reduced thermal stability towards DNA ($\Delta T_m = -4$ °C) when compared to the DNA reference **ON1** (Table 1).[6] When three α-L-RNA monomers were incorporated (**ON4**), the destabilization against the RNA target was limited to −16 °C, whereas no co-operative transition above 5 °C could be detected against DNA.[6] 10-mer ONs composed of a mixture of α-L-RNA monomers and affinity enhancing α-L-LNA[2,3] monomers (**ON13** and **ON14**) displayed efficient hybridization to the corresponding RNA complement ($\Delta T_m = +10$ and +8 °C, respectively), whereas no hybridization towards the corresponding DNA complement could be detected under the applied conditions (Table 2).[6] Moreover, the stability of α-L-RNA/α-L-LNA chimera (**ON13** and **ON14**) towards 3′-exonucleolytic degradation in vitro (snake venom phosphodiesterase) is significantly improved relative to the unmodified DNA reference.[6] If the pronounced RNA selectivity obtained for **ON14** turns out to be a general feature of α-L-RNA/α-L-LNA chimeras, one may envision improved specificity compared to the current antisense molecules, which are known also to hybridize towards DNA targets. A similar pronounced RNA selectivity has been reported for a few other ON analogues, for example, β-L-DNA,[7] arabinonucleic acids,[8] 2′-*O*,3′-*C* linked bicyclic oligonucleotides,[9] and α-D-LNA.[10] However, their usefulness as antisense molecules is hampered either by comparatively low binding affinity toward RNA[7,8] or the necessity of using fully modified oligomers in order to obtain efficient RNA binding.[9,10] The results obtained with thymine α-L-RNA/α-L-LNA chimeras,[6] that is, high binding affinity, RNA-selective hybridization and serum stability motivated us to further investigate this class of RNA stereoisomers.

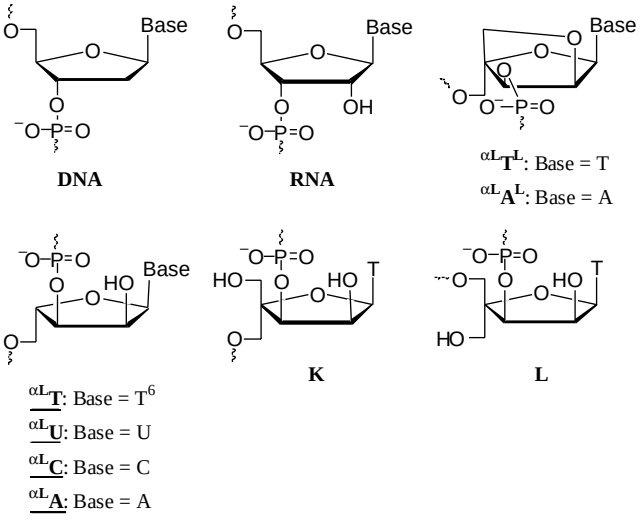


Figure 1. Structures of DNA, RNA, α-L-RNA ($^{\alpha L}$**T**, $^{\alpha L}$**U**, $^{\alpha L}$**C** and $^{\alpha L}$**A**), α-L-LNA ($^{\alpha L}$**T**^{L} and $^{\alpha L}$**A**^{L}),[2,3] 4′-*C*-hydroxymethyl-α-L-RNA (**K**) and 4′-*C*-hydroxymethyl-β-D-lyxofuranosyl (**L**) monomers. The short notations shown are used in Tables 1 and 2. T=thymin-1-yl, U=uracil-1-yl, C=cytosin-1-yl, A=adenin-9-yl.

Table 1. Thermal denaturation experiments[a]

		DNA	RNA
		T_m (ΔT_m) (°C)	T_m (ΔT_m) (°C)
ON1	5′-d(GTGATATGC)	30[b]/28[c] (Ref)	28[b]/26[c]/31[d] (Ref)
ON2	5′-r(GUGAUAUGC)	26 (Ref)	36 (Ref)
ON3	5′-d(GTGA($^{\alpha L}$**T**)ATGC)	26 (−4)[b]	28 (±0)[b]
ON4	5′-d(G($^{\alpha L}$**T**)GA($^{\alpha L}$**T**)A($^{\alpha L}$**T**)GC)	nt[b]	12 (−16)[b]
ON5	5′-d(GTGA**K**ATGC)	23 (−5)[c]	26 (±0)[c]
ON6	5′-d(G**K**GA**K**A**K**GC)	nt[d]	13 (−18)[d]
ON7	5′-d(GTGA**L**ATGC)	24 (−4)[c]	26 (±0)[c]
ON8	5′-d(G**L**GA**L**A**L**GC)	nt[c]	21 (−5)[c]
ON9	5′-r(GUGA**L**AUGC)	25 (−1)[c]	36 (±0)[c]
ON10	5′-r(G**L**GA**L**A**L**GC)	20 (−6)[c]	34 (−2)[c]

[a] Melting temperatures (T_m values) were obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in either medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0)[b,c] or in high salt buffer (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0)[d] using 1.5[b]/1.0[c] μM concentrations of the two complementary strands (assuming identical extinction coefficients for all modified and unmodified nucleotides); ΔT_m values are changes in the T_m value relative to the unmodified reference duplex (Ref); A=adenin-9-yl monomer, C=cytosin-1-yl monomer, G=guanin-9-yl monomer, T=thymin-1-yl monomer, U=uracil-1-yl monomer; see Figure 1 for the structures of α-L-RNA nucleotide monomer ($^{\alpha L}$**T**), 4′-*C*-hydroxymethyl-α-L-RNA thymine monomer **K** and 4′-*C*-hydroxymethyl-β-D-lyxofuranosyl thymine monomer **L**; 'nt'-No co-operative melting transition; DNA sequences are shown as d(sequence) and RNA sequences are shown as r(sequence).

[b] Ref. 6.

This report is focused on the synthesis of the α-L-RNA monomers of three of the naturally occurring RNA monomers ($^{\alpha L}$**U**, $^{\alpha L}$**C** and $^{\alpha L}$**A**) (Fig. 1), their incorporation into

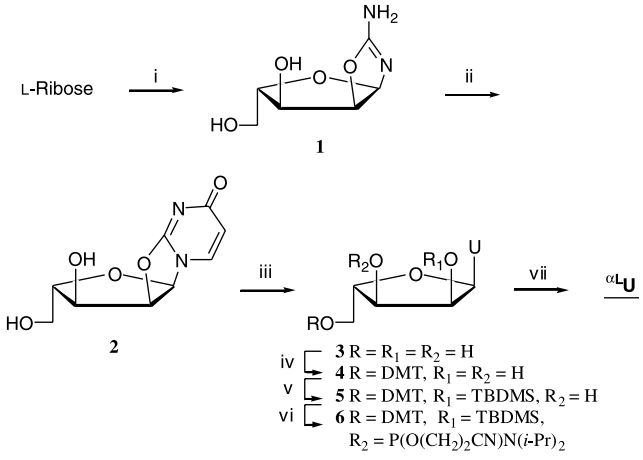
Table 2. Thermal denaturation experiments[a]

		DNA	RNA
		T_m (ΔT_m) (°C)	T_m (ΔT_m) (°C)
ON11	5′-T_{10}	20 (Ref)[b]	19 (Ref)[b]
ON12	5′-T_{14}	30 (Ref)[c]	28 (Ref)[c]
ON13	5′-($^{\alpha L}$**T**)$_4$($^{\alpha L}$**T**^{L})$_4$($^{\alpha L}$**T**)T	nt	29 (+10)[b]
ON14	5′-[($^{\alpha L}$**T**)($^{\alpha L}$**T**)(^{L})]$_4$($^{\alpha L}$**T**)T	nt	27 (+8)[b]
ON15	5′-T_5($^{\alpha L}$**T**)$_4T_5$	nt	11 (−17)[c]
ON16	5′-GTCTCTATGGACCT	45 (Ref)[c]	49 (Ref)[c]
ON17	5′-GTCTCTA($^{\alpha L}$**U**)GGACCT	41 (−4)[c]	47 (−2)[c]
ON18	5′-GTC($^{\alpha L}$**U**)CTATGGACCT	36 (−9)[c]	47 (−2)[c]
ON19	5′-G($^{\alpha L}$**U**)CTCTATGGACCT	40 (−5)[c]	47 (−2)[c]
ON20	5′-ATTATTATAAATTA	32 (Ref)[c]	24 (Ref)[c]
ON21	5′-$^{\alpha L}$(**A**^{L}**T**^{L}**U**^{L}**U**^{L}**T**^{L}**A**^{L}**U**^{L}**A**^{L}**A**^{L}**T**^{L}**T**^{L})A	nt	29 (+5)[c]
ON22	5′-TATTTACTTTC	23 (Ref)[c,d]	26 (Ref)[c,d]
ON23	5′-$^{\alpha L}$(**UA**^{L}**UT**^{L}**UA**^{L}**CT**^{L}**UT**^{L})C	nt	16 (−10)[c,d]
ON24	5′-T_7**L**T_6	21 (−9)[c]	23 (−5)[c]

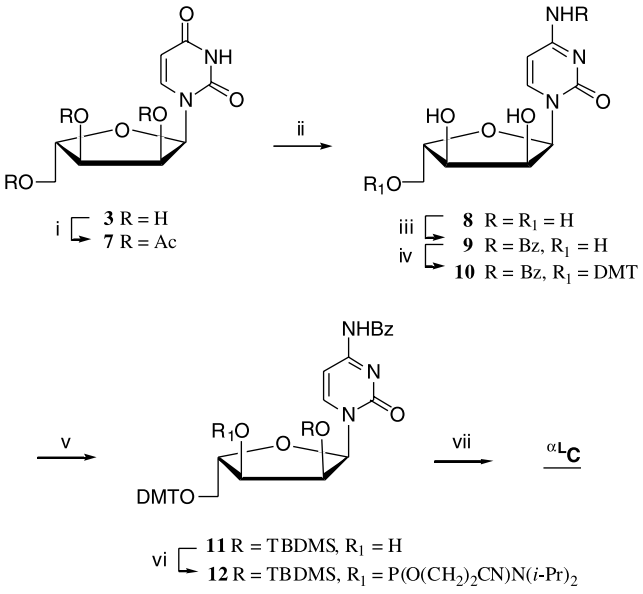
[a] Melting temperatures (T_m values) were obtained from the maxima of the first derivatives of the melting curves (A_{260} vs temperature) recorded in either medium salt buffer[b,c] or in high salt buffer (10 mM sodium phosphate, 1 M sodium chloride, 0.1 mM EDTA, pH 7.0)[d] using 1.5[b]/1.0[c] μM concentrations of the two complementary strands; see below Table 1 for other details; see Figure 1 for the structures of α-L-RNA nucleotide monomers ($^{\alpha L}$**T**, $^{\alpha L}$**U**, $^{\alpha L}$**C** and $^{\alpha L}$**A**), α-L-LNA monomers ($^{\alpha L}$**T**^{L} and $^{\alpha L}$**A**^{L}) and 4′-*C*-hydroxymethyl-β-D-lyxofuranosyl thymine monomer **L**; [d]DNA target [5′-d(AAAGTAAATA)] and RNA target [5′-r(AAAGUAAAUA)] containing a sequence complementary to the first ten monomers of **ON22** and **ON23** were used.

[b] Ref. 6.

oligonucleotides and the study of the stability of duplexes formed between these oligonucleotides and their complementary RNA and DNA strands. The enantiomeric α-D-ribonucleosides derived from uracil, cytosine and adenine have been described previously[11,12] but the low yields reported and the difficult separation of the anomeric mixture in the case of the adenine derivative make these strategies generally unsuitable for the preparation of the enantiomeric α-L-ribonucleosides. Moreover, the utilization of D-ribose as a starting material in these published strategies stimulated us, because of the high cost of L-ribose, to reconsider the strategies for the preparation of the phosphoramidite derivatives (Schemes 1–3).



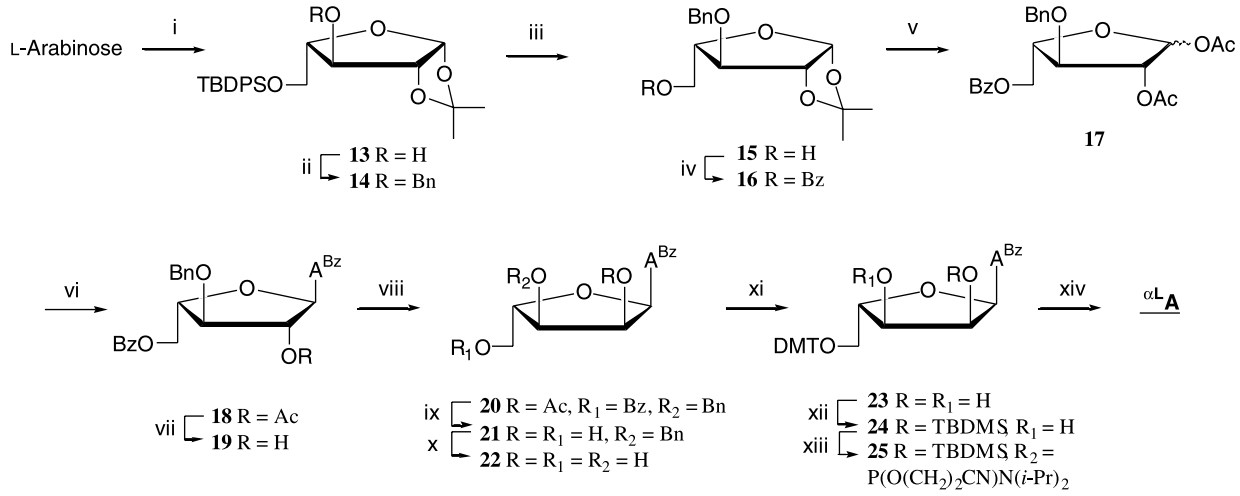
Scheme 1. Reagents and conditions (and yields): (i) NH_2CN, K_2CO_3, DMF, 90 °C (82%); (ii) methyl propiolate, EtOH, reflux (81%); (iii) aq HCl (0.2 N), reflux (77%); (iv) DMTCl, pyridine, rt; (v) TBDMSCl, imidazole, pyridine, rt (42% from **3**); (vi) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (70%); (vii) DNA synthesizer.



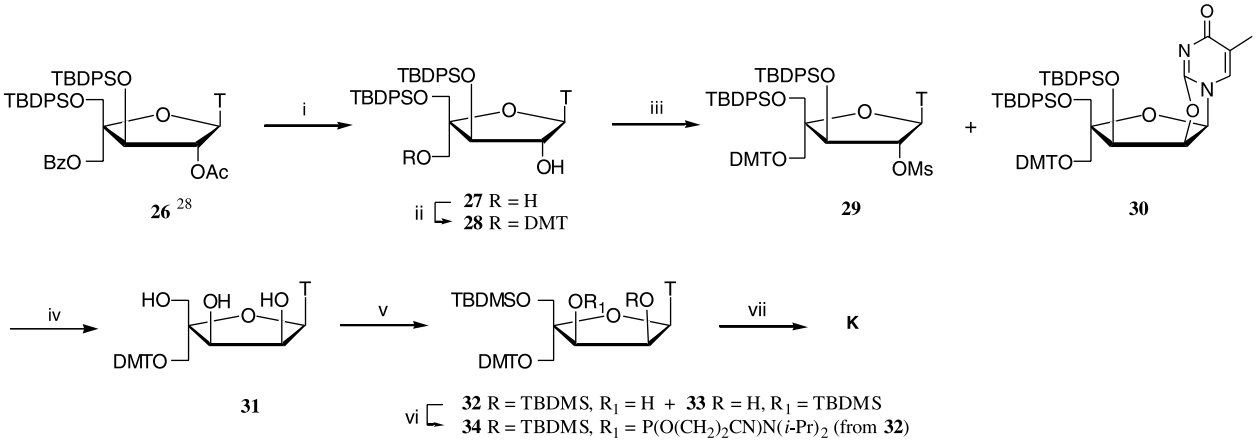
Scheme 2. Reagents and conditions (and yields): (i) Ac_2O, pyridine, rt (83%); (ii) (a) Lawesson's reagent, 1,2-dichloroethane, reflux, (b) saturated methanolic NH_3, 100 °C (74%); (iii) (a) TMSCl, pyridine, rt, (b) BzCl, rt, (c) aq NH_3, rt (77%); (iv) DMTCl, pyridine, rt (95%); (v) TBDMSCl, imidazole, pyridine, rt (49%); (vi) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (52%); (vii) DNA synthesizer.

ONs containing 4′-*C*-hydroxymethyl nucleotide monomers hybridize with both complementary DNA and RNA with virtually identical or slightly improved binding affinity compared to the unmodified duplexes.[13,14] The additional *C*-alkyl branch faces the minor groove for β-D-*ribo*-configured derivatives allowing attachment of molecular entities,[14,15] for example, intercalators, lipophilic groups, positive charged amines or a third strand to an ON. Furthermore, ONs containing C4′-substituted nucleotides have shown increased resistance towards enzymatic degradation.[13,16,17] In order to investigate the influence of the 4′-*C*-hydroxymethyl moiety of α-L-*ribo*-configured monomer **K** on the hybridization towards DNA and RNA complements, we synthesized phosphoramidite **34** and incorporated it into ONs (Scheme 4). The structural resemblance of the flexible monocyclic monomer **K** to the bicyclic α-L-LNA thymine monomer $^{\alpha L}$**T**^{L} added to its interest (Fig. 1).

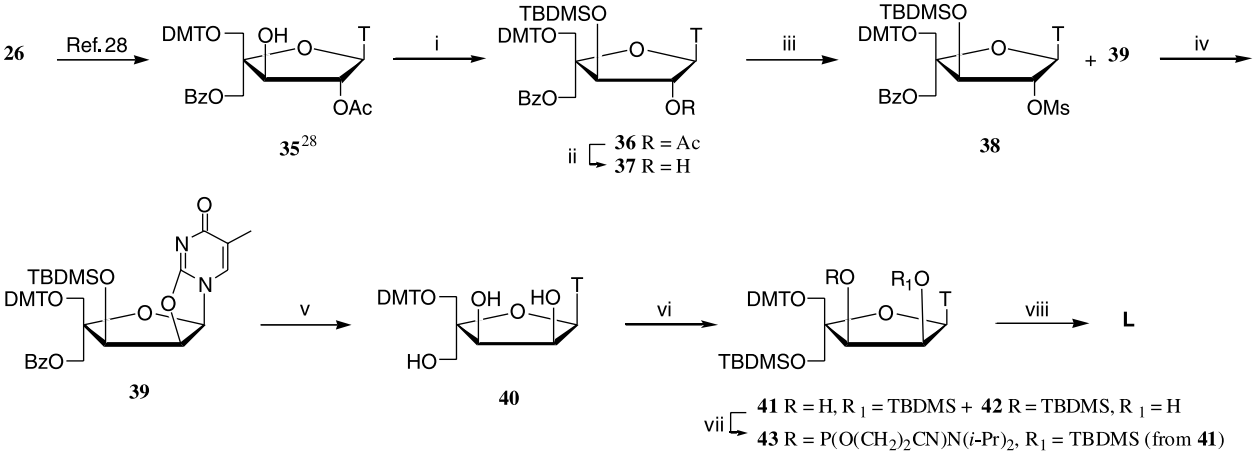
During the recent years a plethora of sugar-modified nucleoside analogues has been chemically synthesized with the aim of improving nucleic acid recognition. However, lyxofuranosyl nucleosides, containing all the three hydroxyls in the 'up' position, have received rather limited attention. Thus, there are only a few reports in the literature regarding their synthesis,[12,18] biological evaluation[19] and conformational investigation.[20] And, to the best of our knowledge, no attempts have been made to incorporate a lyxofuranosyl nucleotide monomer into an ON with the aim of evaluating its hybridization properties. To promote such investigation we describe here the synthesis of phosphoramidite **43** (Scheme 5), starting from the common intermediate **26**, required for the incorporation of 4′-*C*-hydroxymethyl-β-D-lyxofuranosyl thymine monomer **L** into ONs (Fig. 1).



Scheme 3. Reagents and conditions (and yields): (i) two steps (Ref. 24); (ii) BnBr, NaH (60% in mineral oil), DMF, 0 °C to rt (86%); (iii) TBAF, THF, rt (80%); (iv) BzCl, pyridine, rt (74%); (v) (a) aq AcOH (80%), cat. H_2SO_4, rt, (b) Ac_2O, pyridine, rt (73%); (vi) 6-*N*-benzoyladenine, $SnCl_4$, CH_3CN, rt (88%); (vii) half-saturated methanolic NH_3, 0 °C (92%); (viii) (a) Tf_2O, pyridine, CH_2Cl_2, −30 °C, (b) KOAc, 18-crown-6 ether, toluene, CH_2Cl_2, reflux (79%); (ix) aq NaOH (1 M), EtOH, pyridine, 0 °C; (x) Pd/C, HCO_2NH_4, abs EtOH, reflux (54% from **20**); (xi) DMTCl, pyridine, rt (92%); (xii) TBDMSCl, imidazole, pyridine, rt (48%); (xiii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (73%); (xiv) DNA synthesizer.



Scheme 4. Reagents and conditions (and yields): (i) saturated methanolic NH_3, rt (96%); (ii) DMTCl, pyridine, rt, (97%); (iii) MsCl, DMAP, Et_3N, CH_2Cl_2, rt; (iv) aq NaOH (2 M), EtOH, H_2O, reflux (65% from **28**); (v) TBDMSCl, imidazole, pyridine, rt (**32**: 58% and **33**: 23%); (vi) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (71%); (vii) DNA synthesizer.



Scheme 5. Reagents and conditions (and yields): (i) TBDMSCl, imidazole, DMAP, DMF, 36 °C; (ii) saturated methanolic NH_3, MeOH, rt (80% from **35**); (iii) MsCl, Et_3N, DMAP, CH_2Cl_2, rt; (iv) DBU, CH_3CN, rt (83% from **37**); (v) aq NaOH (2 M), EtOH–H_2O (1/1), reflux (74%); (vi) TBDMSCl, imidazole, pyridine, rt (**41**: 36% and **42**: 53%); (vii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2, rt (66%); (viii) DNA synthesizer.

2. Results and discussion

2.1. Synthesis of α-L-ribonucleosides 3, 9 and 22

α-L-Uridine was synthesized following a slightly modified procedure reported for the synthesis of the corresponding D-enantiomer.[11] In our hands, the synthesis of the oxazoline **1** was more efficient with potassium bicarbonate in DMF than with aqueous ammonia as previously described.[11] Oxazoline **1** was obtained in 82% yield from L-ribose. Reaction of **1** with methyl propiolate afforded the anhydro derivative **2** in 81% yield. Subsequent opening of this ring with acid under aqueous conditions afforded the desired α-L-uridine **3** in 77% yield. The ^{1}H NMR data of **3** were found to be consistent with the literature data for the corresponding D-enantiomer (Scheme 1).[12]

Compound **3** was per-acetylated with acetic anhydride in pyridine to give **7** in 83% yield. Synthesis of α-D-cytidine has been reported[12b] in a moderate 48% yield applying the Sung methodology[21] to furnish the D-enantiomer of **8**. Nevertheless, reaction of **7** with the Lawesson's reagent[22] followed by treatment with saturated methanolic ammonia afforded α-L-cytidine **8** in 74% yield. Using the transient protection method,[23] α-L-cytidine was *N*-benzoylated to afford nucleoside **9** in 77% yield. The ^{1}H NMR spectroscopic data for compounds **8** and **9** were found to be consistent with the literature data for their D-enantiomers (Scheme 2).[12b]

Using inexpensive L-arabinose as starting material, furanose **13** was prepared in a two step procedure developed by Dahlman et al.[24] The 3-hydroxy group of **13** was benzylated using benzyl bromide and sodium hydride in DMF to give furanose **14** in 86% yield. Cleavage of the silyl protecting group of **14** using TBAF gave derivative **15** (80% yield) that was benzoylated to give furanose **16** in 74% yield. The ^{1}H NMR spectroscopic data of furanoses **14** and **15** were consistent with the literature data for their D-enantiomer.[25] Finally, the glycosyl donor **17** was obtained by a standard two step procedure of isopropylidene group cleavage and acetylation of the 1- and 2-hydroxy functions in 73% overall yield. Furanose **17** was condensed with *N*-6-benzoyladenine under the conditions initially reported by Saneyoshi and Satoh[26] where the base is directly reacted with the *O*-acetylated sugar in the presence of stannic chloride. Coupling proceeded in 88% yield to afford nucleoside **18**. It can be noticed that the participation of the 2′-*O*-acetyl group provided only the desired α-anomer. Compound **18** was selectively deprotected at the 2′-position by the action of half-saturated methanolic ammonia to give nucleoside **19** in good yield (92%). Inversion of the configuration at C2′ proceeded in two steps. Firstly, the 2′-hydroxy group was activated by reaction with triflic anhydride followed by the reaction of the intermediate with potassium acetate to give the expected inverted nucleoside **20** in 79% yield. Nucleoside **20** was selectively deacylated at the 2′- and 5′-positions with aqueous sodium hydroxide in an ethanol–pyridine mixture following a previously described procedure[27] to give nucleoside **21** in 77% yield. Finally, debenzylation of compound **21** by treatment with ammonium formate and palladium on carbon produced *N*-6-benzoyl-α-L-adenosine **22** in a yield of 70% (Scheme 3). The ^{1}H NMR spectroscopic data of nucleoside **22** were consistent with the literature data for its D-enantiomer.[12b]

2.2. Synthesis of the α-L-RNA phosphoramidites 6, 12 and 25

Compounds **3**, **9** or **22** were O5′-dimethoxytritylated using standard conditions in satisfactory yields (95, 95 and 92%, respectively). Silylation of nucleosides **4**, **10** or **23** with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole and pyridine produced a mixture of a byproduct (fast eluting; assigned as the 2′-*O*-TBDMS isomers) and 3′-*O*-TBDMS (slow eluting) derivatives, which were separated by silica gel column chromatography. The ^{1}H NMR spectral data obtained for compounds **5**, **11** or **24** were found to be consistent with the literature data for their D-enantiomers.[12] Nucleosides **5**, **11** or **24** were dissolved in anhydrous dichloromethane and phosphitylated using 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite in the presence of *N*,*N*-diisopropylethylamine to give the corresponding phosphoramidites **6**, **12** and **25** in yields of 70, 52 and 73%, respectively.

2.3. Synthesis of the 1-(4-*C*-hydroxymethyl-α-L-ribofuranosyl)thymine phosphoramidite 34

Complete deacylation of 1-[2-*O*-acetyl-3,5-(di-*O*-*tert*-butyldiphenylsilyl)-4-*C*-benzoyloxymethyl-β-D-xylofuranosyl]thymine (**26**)[28] with saturated methanolic ammonia, followed by regioselective dimethoxytritilation afforded nucleoside **28**. Activation of the 2′-OH in nucleoside **28** by reaction with MsCl afforded the desired nucleoside **29** along with the 2,2′-anhydro nucleoside **30**. This crude mixture was refluxed under alkaline conditions, which also resulted in complete desilylation, affording triol **31**. Silylation of the triol **31** with 4 equiv of TBDMSCl afforded the desired 2′-*O*-TBDMS isomer **32** in 58% yield along with the 3′-*O*-isomer **33** (23% yield). Phosphitylation of **32** by the standard protocol afforded phosphoroamidite **34** (71% yield) that was used to incorporate monomer **K** into ONs (Scheme 4). To ascertain that no silyl migration occurred under the basic conditions applied during phosphitylation, the isomers of **34** were separated and characterized. The signals of H3′ appeared as a double doublet with a large $^2J_{H,P}$ coupling constant (major isomer: 13.7 Hz; minor isomer: 13.0 Hz) confirming that no silyl migration occurred during the course of the reaction.[29]

2.4. Synthesis of the 1-(4-*C*-hydroxymethyl-β-D-lyxofuranosyl)thymine phosphoramidite 43

Selective removal of the primary silyl protection in the common intermediate **26** proved difficult, probably due to silyl migration from the 3′-position. Therefore, complete desilylation of nucleoside **26** and subsequent O5′-tritylation followed by O3′-silylation furnished nucleoside **36** in 66% overall yield (from **26** via **35**). Deacetylation afforded nucleoside **37**, which upon mesylation yielded a mixture of nucleosides **38** and **39** (~3:1, as judged from analytical TLC). Concomitant treatment with aq NaOH furnished a complex mixture, presumably via desilylalation of **38**, followed by epoxide formation and then opening of the epoxide under alkaline conditions. The desired *lyxo*-configured nucleoside **40** was obtained by complete conversion of the crude mixture (**38**+

39) into O2′,C2-anhydronucleoside **39**, followed by treatment with aq NaOH in ethanol, affording nucleoside **40** in 61% overall yield (from **37**). Silylation of the triol **40** by reaction with 4 equiv of TBDMSCl afforded the desired 2′-*O*-TBDMS isomer **41** in 36% yield along with the O3′-isomer **42** (52%). O3′-phosphitylation of nucleoside **41** afforded phosphoroamidite **43** (66% yield) that was used to incorporate monomer **L** into ONs (Scheme 5). The signal of H3′ in **43** appeared as a double doublet with a large $^{2}J_{H,P}$ coupling constant (major isomer: 13.6 Hz) indicating that no silyl migration occurred during phosphitylation.

3. Synthesis of ONs and thermal denaturation studies

All oligomers **ON5**–**ON10**, **ON15**, **ON17**–**ON19**, **ON21**, **ON23** and **ON24** (Tables 1 and 2) were prepared in 0.2 μmol scale using the phosphoramidite approach (see the Section 7 for details). The composition of the oligomers was verified by MALDI-MS analysis (see the Section 7) and their purity (>80%) by capillary gel electrophoresis.

Results from hybridization experiments (T_m values) towards single-stranded DNA and RNA complements are shown in Tables 1 and 2. A single replacement of a DNA thymine monomer in a 9-mer mixed-base sequence by its α-L-RNA counterpart $^{\alpha L}$**T** resulted in destabilization of the duplex by 4 °C when hybridized to complementary DNA (Table 1, **ON3** relative to **ON1**), while no change in the duplex stability was seen when hybridized to the RNA complement.[6] Incorporation of a few isolated $^{\alpha L}$**T** monomers into a DNA strand reduced the affinity towards the RNA target ($\Delta T_m = -16$ °C, **ON4** relative to **ON1**), but the effect was more pronounced towards the DNA target (no co-operative transition above 5 °C could be detected).[6] A single incorporation of an α-L-RNA U monomer in a 14-mer mixed-base sequence **ON18** leads to a decrease in duplex stability against the DNA target ($\Delta T_m = -9$ °C) when compared to the DNA reference **ON16**; the effect is less detrimental when the substitution is either in the centre (**ON17**, $\Delta T_m = -4$ °C) or towards the 5′-end (**ON19**, $\Delta T_m = -5$ °C). However, against the RNA complement the stability ($T_m = 47$ °C, $\Delta T_m = -2$ °C) was comparable with that of the DNA·RNA reference duplex. The fact that incorporation of a single α-L-RNA monomer is tolerated in a duplex with complementary RNA is likely explained by conformational adaptation that is impossible for the relatively short duplexes following incorporation of more than one α-L-RNA monomer. The stereoregular (almost) fully modified α-L-RNA/α-L-LNA chimera **ON13**,[6] **ON14**[6] and **ON21** consisting of a mixture of α-L-RNA and α-L-LNA monomers displayed very efficient hybridization towards the RNA target ($\Delta T_m = +10$, $+8$ and $+5$ °C, respectively), whereas no hybridization towards the DNA target was detected. It should be noted that **ON15**, having four consecutive α-L-RNA T monomers and no α-L-LNA monomer, displayed significantly decreased affinity towards the RNA complement. Similar RNA-selective hybridization was seen with the α-L-RNA/α-L-LNA chimera **ON23** consisting of alternate α-L-RNA and α-L-LNA monomers. Thus, although no co-operative transition could be detected at medium salt conditions, a melting temperature was observed against the RNA target under high salt conditions.

A single incorporation of 1-(4-*C*-hydroxymethyl-α-L-ribofuranosyl)thymine monomer **K** in the middle of a 9-mer mixed-base sequence induced similar hybridization properties as incorporation of the α-L-RNA thymine monomer $^{\alpha L}$**T** (**ON5** compared to **ON3**), that is, a decrease in T_m value (−5 °C) against the DNA complement and no change against the RNA complement. The partly modified 9-mer containing three incorporations of monomer **K** induced a similar destabilizing effect ($\Delta T_m = -6$ °C/modification, **ON6** relative to **ON1**) on the duplex formed with complementary RNA as seen above with **ON4** containing three $^{\alpha L}$**T** monomers ($\Delta T_m = -5.3$ °C/modification, **ON4** relative to **ON1**), indicating no unfavorable steric hindrance due to the additional 4′-*C*-alkyl chain.

The 4′-*C*-hydroxymethyl-β-D-lyxofuranosyl thymine monomer **L**, containing all three hydroxyls in 'up' position, displayed some interesting hybridization properties. A single incorporation of monomer **L** in a 9-mer mixed-base sequence **ON7** showed preference for binding to its RNA complement (T_m unchanged) relative to its DNA complement ($\Delta T_m = -4$ °C). However, the duplex stability decreased when the modification was placed in the centre of a 14-mer homopyrimidine sequence (**ON24**, $\Delta T_m = -9$ °C against the DNA target and $\Delta T_m = -5$ °C against the RNA target). A T_m value of 21 °C was observed with the RNA complement hybridized to the partly modified stereoirregular 9-mer mixed-base sequence **ON8**, but no co-operative transition above 5 °C was observed with the DNA complement. In contrast, efficient recognition of both DNA and RNA targets was achieved when monomer **L** was incorporated into an RNA strand, and satisfactory binding affinity towards DNA and RNA complements was observed with one (**ON9**) and three (**ON10**) incorporations.

4. Molecular modeling

Molecular modeling (see the Section 7 for details) was used to rationalize the thermal stability results for monomers

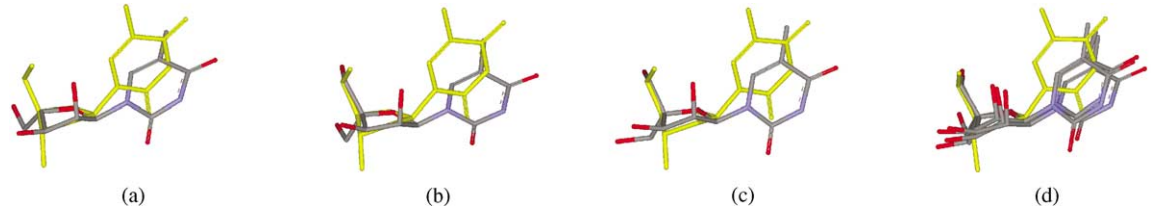


Figure 2. ON:DNA duplexes: (a) the tilted base is indicated for the overlaid structures of standard DNA thymine monomer (yellow) and $^{\alpha L}$**T** monomer; (b) overlaid structures of DNA (T) and **K** monomers; (c) overlaid structures of DNA (T) and **L** monomers; (d) the *S*-type furanose conformation is shown for standard DNA (yellow) and $^{\alpha L}$**T**, **K**, and **L** monomers (all monomer structures have been cut out of the corresponding duplex helix structure and overlaid with the sugar ring system kept fixed).

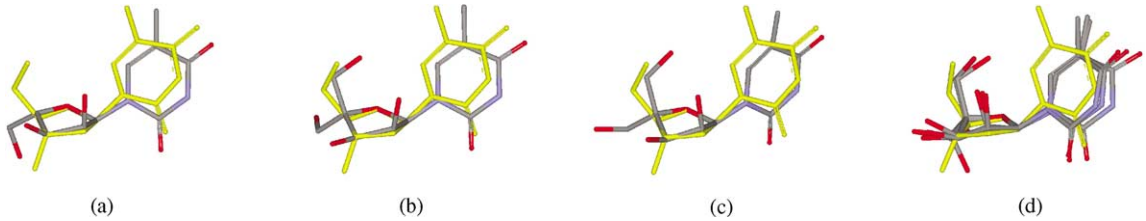


Figure 3. ON:RNA duplexes. See legend to Figure 2 for further details.

$^{\alpha L}$**T**, **K** and **L**. All monomers showed S-type furanose conformations[†] in both ON·DNA (Fig. 2) and ON·RNA (Fig. 3) hybrids. The most pronounced change was the tilting of the nucleobase in all the three modified monomers when compared to the reference DNA (T) monomer (Table 3).

Table 3. Torsion angle χ (O4′–C1′–N1–C2)

Modification	Torsion angle χ (°)	
	[ON:DNA]	[ON:RNA]
DNA-T (Ref)	−96	−135
$^{\alpha L}$**T**	−168	−156
K	−169	−152
L	−151	−97

The calculated structures for monomers $^{\alpha L}$**T**, **K**, and **L** are in agreement with the observed thermal stabilities. Single incorporations of the monomers $^{\alpha L}$**T**, **K**, or **L** in a 9-mer ON leads to reduced affinity towards the complementary DNA target, which can be attributed to significant change in the torsion angle χ in the modified monomers (Table 3). The base displacement causes reduced stacking within the strand and a loss of hydrogen bonding towards the complementary base. However, in duplexes with the RNA complement the change in the torsion angle χ in monomers $^{\alpha L}$**T**, **K**, and **L** (compared to DNA-T monomer) is more limited, which offers an explanation for the RNA-selective hybridization induced by the incorporation of these monomers.

5. RNase H cleavage

Stimulated by the satisfactory RNA binding characteristics of the α-L-RNA modified ONs, we studied RNase H degradation of [^{32}P] labelled RNA that was complementary to **ON16–ON19**. Hybridized samples were digested for different time intervals and the RNA was electrophoresed on an acryamide gel and visualized by autoradiography. Basic hydrolysis of RNA (Fig. 4) was used to identify the cleaved positions. As can be seen in Figure 4, the unmodified reference **ON16** mainly supports RNase H cleavage at phosphodiester bonds opposite positions 4–5, 6–7 and 7–8. **ON17** that is modified at position 8 is less efficiently cleaved than **ON16** showing no 4–5 cleavage band, a weak 5–6 band and a strong 7–8 band. This indicates that the modification interferes moderately with initial binding of RNase H but also shows that the enzyme can cleave opposite 5′ to the modification. **ON18** modified at position 4 provides an even better cleavage than the reference **ON16** with cleavage mainly opposite positions 6–7 and 7–8. The cleavage pattern for **ON19** modified at position 2 is very similar to the pattern seen with the reference **ON16**. The RNA complement can thus be cleaved both to the 3′ and 5′ site of α-L-RNA residues in the corresponding oligonucleotide, showing that RNase H cleavage can take place in close proximity to an α-L-RNA monomer. These results show that properly designed α-L-RNA/DNA mixmers can be attractive molecules for antisense applications.

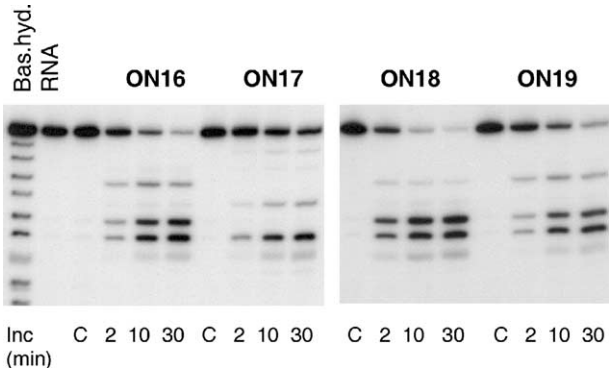


Figure 4. Autoradiogram showing gel electrophoresis of RNase H cleavage of labelled RNA hybridized to complementary **ON16–ON19**. **C** are hybridized samples incubated in the absence of RNase H.

6. Conclusion

Oligonucleotides containing α-L-RNA monomers display in general decreased duplex stability relative to the unmodified reference duplexes, but at the same time preferential binding towards the RNA complement. Despite the unnatural configuration of the α-L-RNA monomer, DNA ONs containing a single incorporation of an α-L-RNA monomer retain the ability to elicit RNase H activity. Moreover, increased binding affinity and RNA-selective hybridization was induced by combining the α-L-RNA monomers with affinity enhancing α-L-LNA monomers. As furthermore the α-L-RNA/α-L-LNA chimeras displayed significant stabilization towards 3′-exonucleolytic degradation,[6] these classes of molecules are excellent candidates for use within the antisense technology. The presence of a 4′-*C*-alkyl group in 4′-hydroxymethyl-α-L-RNA monomer **K** had no influence on the duplex stability when compared to the α-L-RNA monomer, and could therefore function as a handle for the attachment of amino functionalities to improve the binding affinity or the pharmacokinetic properties of ONs containing α-L-RNA monomers. RNA-selective hybridization was also achieved by the incorporation of 1-(4-*C*-hydroxymethyl-β-D-lyxofuranosyl)thymine monomer **L** into a DNA strand,

whereas stable duplexes towards both complementary DNA and RNA were formed upon incorporation of monomer **L** into an RNA strand.

7. Experimental

7.1. General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica plates with fluorescence indicator (SiO_2-60, F-254) visualizing under UV light and by revelation with 5% concd sulfuric acid in ethanol (v/v) followed by heating. Silica gel 60 (particle size 0.040–0.063 mm, Merck) was used for flash column chromatography. Light petroleum of the distillation range 60–80 °C was used. After column chromatography fractions containing product were pooled, evaporated to dryness under reduced pressure and dried for 12 h under vacuum to give the product unless otherwise specified. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75.5 MHz, and ^{31}P NMR spectra at 121.5 MHz. Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as internal standard for ^{1}H and ^{13}C NMR, and relative to 85% H_3PO_4 as external standard for ^{31}P NMR. Assignments of NMR spectra, when given, are based on 2D spectra and follow the standard carbohydrate/nucleoside nomenclature (the carbon atom of the C-4′-subsituent is numbered C5″). The assignments of methylene protons, when given, may be interchanged. Coupling constants (*J* values) are given in Hertz. MALDI-HRMS were recorded in positive ion mode on an IonSpec Fourier Transform mass spectrometer.

7.1.1. 2-Amino-α-L-ribofurano[1′,2′:4,5]-2-oxazoline (1). A mixture of L-ribose (2.00 g, 13.3 mmol), cyanamide (0.67 g, 16.0 mmol) and powdered potassium bicarbonate (0.07 g, 0.05 mmol) was stirred at 90 °C for 1 h in anhydrous DMF (15 mL). After cooling to room temperature, the mixture was evaporated under reduced pressure to half volume and the resulting solution was stored for 20 h at 5 °C. The precipitate obtained was filtered off and recrystallized from 96% aq EtOH to give 1.90 g of oxazoline **1** (82%) as a white solid material. δ_H (DMSO-d_6) 6.26 (2H, br s, NH_2), 5.58 (1H, d, J=4.8 Hz, H1′), 5.17 (1H, br s, OH), 4.59–4.56 (2H, m, H2′ and OH), 3.74–3.63 (2H, m, H3′ and H4′), 3.42–3.25 (2H, m, H5′); δ_C (DMSO-d_6) 163.8, 98.3, 80.8, 77.8, 71.2, 60.4; MALDI-MS: *m/z* 197 ($[M+Na]^+$, $C_6H_{10}N_2O_4Na^+$ calcd 197).

7.1.2. 2,2′-Anhydro-1-(α-L-ribofuranosyl)uracil (2). A mixture of oxazoline **1** (1.00 g, 5.75 mmol) in 96% aq EtOH (10 mL) and methyl propiolate (1.69 g, 20.1 mmol) was heated under reflux for 2 h. After cooling to room temperature, the reaction mixture was evaporated to dryness under reduced pressure and then coevaporated several times with 96% aq EtOH to give 1.05 g of nucleoside **2** as a white solid material (81%) after recrystallization from EtOH. δ_H (DMSO-d_6) δ 7.85 (1H, d, J=7.4 Hz, H6), 6.20 (1H, d, J=5.2 Hz, H1′), 5.88 (1H, d, J=7.4 Hz, H5), 5.74 (1H, d, J=6.9 Hz, 3′-OH), 5.23 (1H, t, J=5.2 Hz, H2′), 4.86 (1H, t, J=5.0 Hz, 5′-OH), 4.05 (1H, m, H3′), 3.70 (1H, dd, J=5.0, 12.0 Hz, H5′a), 3.57 (1H, m, H4′), 3.46 (1H, m, H5′b); δ_C (DMSO-d_6) 171.0, 160.7, 136.8, 108.8, 88.6, 81.4, 80.7, 69.8, 59.5; MALDI-MS: *m/z* 249 ($[M+Na]^+$, $C_9H_{10}N_2O_5Na^+$ calcd 249).

7.1.3. 1-(α-L-Ribofuranosyl)uracil (3). A solution of nucleoside **2** (2.17 g, 9.6 mmol) in aqueous hydrochloric acid (0.2 N, 10 mL) was refluxed for 1 h. After cooling to room temperature, the solution was neutralized using Amberlyst IRA 410 [OH^-]. The resin was filtered off and washed with lukewarm H_2O. The combined filtrate was evaporated to dryness under reduced pressure. The residue was purified on a silica gel column, [15% (v/v) MeOH in EtOAc] affording 1.80 g (77%) of nucleoside **3** as a white solid material. δ_C (DMSO-d_6) 163.5, 150.7, 142.8, 99.8, 85.1, 84.0, 70.4, 70.3, 61.2; MALDI-MS: *m/z* 267 ($[M+Na]^+$, $C_9H_{12}N_2O_6Na^+$ calcd 267). The ^{1}H NMR data of were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.4. 1-(5-*O*-(4,4′-Dimethoxytrityl)-α-L-ribofuranosyl)-uracil (4). 4,4′-Dimethoxytrityl chloride (0.43 g, 1.3 mmol) was added to a solution of nucleoside **3** (0.26 g, 1.07 mmol) in anhydrous pyridine (5 mL). The reaction mixture was stirred at room temperature for 12 h whereupon methanol (2 mL) was added. After stirring for additional 10 min, the mixture was poured into saturated aq $NaHCO_3$ (25 mL). Extraction was performed with $CHCl_3$ (3×20 mL), and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [5–8% MeOH in $CHCl_3$ containing 0.5% Et_3N (v/v/v)] to give nucleoside **4** (550 mg) as a white foam. NMR spectroscopic data revealed the compound to be contaminated with traces of Et_3N. δ_C ($CDCl_3$) 164.3, 158.4, 150.9, 144.6, 142.7, 135.8, 135.7, 130.0, 129.9, 128.1, 127.8, 126.8, 113.2, 100.4, 86.6, 86.3, 84.3, 72.0, 71.2, 63.3, 55.2; MALDI-MS: *m/z* 569 ($[M+Na]^+$, $C_{30}H_{30}N_2O_8Na^+$ calcd 569). The ^{1}H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.5. 1-(2-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl)uracil (5). Nucleoside **4** (3.20 g) and imidazole (1.04 g, 15.2 mmol) were dissolved in anhydrous pyridine (60 mL). TBDMSCl (1.15 g, 7.6 mmol) was added and the solution was stirred at room temperature for 24 h. The reaction mixture was then poured into saturated aq $NaHCO_3$ (120 mL) and extraction was performed with $CHCl_3$ (3×80 mL). The combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [5–7% (v/v) acetone in CH_2Cl_2] yielding the 2′-*O*-*tert*-butyldimethylsilyl isomer **5** (2.90 g, 42% from **3**) as a clear oil and [9–10% (v/v) acetone in CH_2Cl_2] a byproduct tentatively assigned as the 3′-*O*-*tert*-butyldimethylsilyl isomer (yield not determined). δ_C ($CDCl_3$) 163.3, 158.7, 150.6, 149.9, 144.5, 142.0, 135.7, 135.4, 130.0, 128.1, 128.0, 127.1, 123.8, 113.4, 113.3, 101.0, 87.0, 85.9, 84.5, 72.8, 72.7, 64.2, 55.3, 25.7, 18.1, −5.2, −5.3; ESI-HRMS: *m/z* 683.2723 ($[M+Na]^+$, $C_{36}H_{44}N_2O_8SiNa^+$ calcd 683.2759). The ^{1}H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12a]

7.1.6. 1-(2-*O*-*tert*-Butyldimethylsilyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl)uracil (6). To a stirred solution of nucleoside **5** (0.26 g, 0.40 mmol) in CH_2Cl_2 (10 mL) at room temperature was added *N*,*N*-diisopropylethylamine (0.69 mL, 3.95 mmol). After dropwise addition of 2-cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (0.38 mL, 1.98 mmol), the reaction mixture was stirred for another 15 h. CH_2Cl_2 (20 mL) was added and the mixture was washed with saturated aq $NaHCO_3$ (25 mL). The organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [45–50% EtOAc in *n*-hexane, containing 0.5% Et_3N (v/v/v)] to yield phosphoramidite **6** as a white foam (240 mg, 70%). δ_P (DMSO-d_6) 151.0, 149.9; ESI-HRMS: *m*/*z* 883.3838 ($[M+Na]^+$, $C_{45}H_{61}N_4O_9PSiNa^+$ calcd 883.3843).

7.1.7. 1-(2,3,5-Tri-*O*-acetyl-α-L-ribofuranosyl)uracil (7). Acetic anhydride (2.32 mL, 24.5 mmol) was added to a solution of nucleoside **3** (1.71 g, 7.0 mmol) in anhydrous pyridine (10 mL). The reaction mixture was stirred at room temperature for 12 h. MeOH (5 mL) was added and the reaction mixture was stirred for another 10 min and then concentrated to dryness under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washing was performed first with saturated aq $NaHCO_3$ (25 mL) and then brine (25 mL). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [3–5% (v/v) MeOH in $CHCl_3$] to afford nucleoside **7** (2.16 g, 83%) as a white foam. δ_H ($CDCl_3$) 9.47 (1H, br s, NH), 7.47 (1H, d, J=8.2 Hz, H6), 6.39 (1H, d, J=4.7 Hz, H1′), 5.77 (1H, d, J=8.1 Hz, H5), 5.71 (1H, t, J=4.9 Hz, H2′), 5.42 (1H, t, J=5.4 Hz, H3′), 4.55 (1H, m, H4′), 4.36 (1H, dd, J=3.2, 12.3 Hz, H5′a), 4.18 (1H, dd, J=4.2, 12.2 Hz, H5′b), 2.15, 2.07 and 2.03 (3H each, 3s, 3×$COCH_3$); δ_C ($CDCl_3$) 170.5, 169.3, 168.7, 163.3, 150.2, 140.2, 101.6, 84.3, 79.9, 70.9, 70.3, 63.1, 20.9, 20.5, 20.4; MALDI-HRMS: *m*/*z* 393.0885 ($[M+Na]^+$, $C_{15}H_{18}N_2O_9Na^+$ calcd 393.0905).

7.1.8. 1-(α-L-Ribofuranosyl)cytosine (8). The Lawesson's reagent (1.80 g, 4.45 mmol) was added to a stirred solution of nucleoside **7** (2.06 g, 5.57 mmol) in anhydrous 1,2-dichloroethane (50 mL). The reaction mixture was heated under reflux for 4 h and then cooled to room temperature. Methanol (20 mL) was added and the reaction mixture concentrated to dryness under reduced pressure. The residue was immediately dissolved in a saturated solution of ammonia in methanol (100 mL) and heated at 100 °C for 3 h in an autoclave. After cooling to room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The residue was purified by column chromatography [5–10% (v/v) MeOH in EtOAc] to give nucleoside **8** (1.0 g, 74%) as a white powder. δ_C (DMSO-d_6) 165.5, 155.2, 143.1, 92.2, 85.6, 83.1, 70.6, 70.1, 61.1; MALDI-MS: *m*/*z* 266 ($[M+Na]^+$, $C_9H_{13}N_3O_5Na^+$ calcd 266). The ^1H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.9. 4-*N*-Benzoyl-1-(α-L-ribofuranosyl)cytosine (9). To a stirred solution of nucleoside **8** (1.0 g, 4.11 mmol) in anhydrous pyridine (20 mL) at 0 °C was added trimethylchlorosilane (3.13 mL, 24.7 mmol). The reaction mixture was stirred at room temperature for 1 h whereupon benzoyl chloride (2.38 mL, 20.6 mmol) was added. After stirring for another 5 h the resulting mixture was cooled in an ice bath, H_2O (10 mL) was added and stirring was continued for additional 5 min. Aqueous ammonia (10 mL, 29%, w/w) was added, and the resulting mixture was stirred at room temperature for 15 min and evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (2×5 mL) and then purified by column chromatography [5–8% (v/v) MeOH in EtOAc] affording nucleoside **9**, which was crystallized from absolute ethanol as colourless crystals (1.1 g, 77%). δ_C (DMSO-d_6) 167.2, 162.9, 154.6, 147.0, 133.2, 132.7, 128.4, 128.2, 95.0, 86.7, 83.7, 70.6, 70.0, 60.9; MALDI-HRMS: *m*/*z* 370.1009 ($[M+Na]^+$, $C_{16}H_{17}N_3O_6Na^+$ calcd 370.1015). The ^1H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.10. 4-*N*-Benzoyl-1-[5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl]cytosine (10). 4,4′-Dimethoxytrityl chloride (0.33 g, 1.0 mmol) was added to a solution of nucleoside **9** (0.12 g, 0.49 mmol) in anhydrous pyridine (5 mL) and the resulting mixture was stirred at room temperature for 12 h. MeOH (2 mL) was added, stirring was continued for another 10 min whereupon the reaction mixture was poured into saturated aq $NaHCO_3$ (25 mL). Extraction was performed with $CHCl_3$ (3×20 mL), and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [6–8% (v/v) MeOH in $CHCl_3$] to furnish nucleoside **10** (180 mg, 95%) as a white foam. δ_C ($CDCl_3$) 166.7, 162.5, 158.6, 156.2, 146.5, 144.7, 136.0, 135.8, 133.2, 133.0, 130.2, 129.1, 128.2, 128.0, 127.8, 127.0, 113.3, 96.3, 88.9, 86.5, 84.5, 72.1, 71.3, 63.8, 55.3; MALDI-MS: *m*/*z* 649 ($[M+Na]^+$, $C_{37}H_{35}N_3O_8Na^+$ calcd 649). The ^1H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.11. 4-*N*-Benzoyl-1-(2-*O*-*tert*-butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl)cytosine (11). *tert*-Butyldimethylsilyl chloride (0.19 g, 1.05 mmol) was added to a solution of nucleoside **10** (0.44 g, 0.81 mmol) and imidazole (0.14 g, 2.10 mmol) in anhydrous pyridine (10 mL) and stirring was continued at room temperature for 24 h. The reaction mixture was poured into a saturated aq $NaHCO_3$ (25 mL) and extraction was performed with $CHCl_3$ (3×20 mL). The combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [5–8% (v/v) acetone in CH_2Cl_2] to give the 2′-*O*-*tert*-butyldimethylsilyl isomer **11** as a white foam (0.30 g, 49%). Further elution [8–10% (v/v) acetone in CH_2Cl_2] yielded a byproduct tentatively assigned as the 3′-*O*-*tert*-butyldimethylsilyl isomer (yield not determined). δ_C ($CDCl_3$) 162.1, 158.7, 146.5, 144.6, 135.8, 135.6, 133.3, 130.1, 129.2, 128.2, 128.1, 127.7, 127.1, 113.4, 95.5, 87.3, 86.9, 84.4, 73.0, 72.7, 64.0, 55.4, 25.9, 18.2, −5.1, −5.3; ESI-MS: *m*/*z* 786 ($[M+Na]^+$, $C_{43}H_{49}N_3O_8SiNa^+$ calcd 786). The ^1H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.12. 4-*N*-Benzoyl-1-(2-*O*-*tert*-butyldimethylsilyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphino]-5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl)cytosine (12). To a stirred solution of nucleoside **11** (70 mg, 0.09 mmol) and *N*,*N*-diisopropylethylamine (0.16 mL, 0.92 mmol) in CH_2Cl_2 (4 mL) at room temperature was added 2-cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (0.09 mL, 0.46 mmol) and stirring was continued for 15 h. CH_2Cl_2 (10 mL) was added and the resulting mixture was washed with saturated aq $NaHCO_3$ (10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [45–50% EtOAc in *n*-hexane, containing 0.5% Et_3N (v/v/v)] affording phosphoramidite **12** (50 mg, 52%) as a white foam. δ_P (DMSO-d_6) 151.4, 150.9.

7.1.13. 3-*O*-Benzyl-5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-β-L-arabinofuranose (14). To a solution of 5-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-β-L-arabinofuranose **13**[24] (7.00 g, 16.4 mmol) in anhydrous DMF (50 mL) at 0 °C was added NaH (1.31 g, 60% suspension in mineral oil, 32.7 mmol) and benzyl bromide (3.9 mL, 32.7 mmol). The reaction mixture was stirred at room temperature for 5 h and then concentrated to dryness under reduced pressure. The residue obtained was dissolved in diethyl ether (100 mL) and washing was performed successively with saturated aq $NaHCO_3$ (100 mL) and brine (100 mL). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [5–10% (v/v) EtOAc in light petroleum] to afford nucleoside **14** as a clear oil (7.20 g, 86%). δ_C ($CDCl_3$) 137.5, 135.6, 135.5, 133.1, 129.7, 128.5, 127.8, 127.7, 127.68, 127.64, 112.4, 105.7, 85.2, 85.1, 82.8, 71.6, 63.4, 26.9, 26.8, 26.1, 19.2; MALDI-HRMS: *m*/*z* 541.2382 ($[M+Na]^+$, $C_{31}H_{38}O_5SiNa^+$ calcd 541.2386). The ^1H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[25b]

7.1.14. 3-*O*-Benzyl-1,2-*O*-isopropylidene-β-L-arabinofuranose (15). To a solution of furanose **14** (9.84 g, 19.0 mmol) in THF (150 mL) was added TBAF (38.0 mL, 1 M in THF, 38.0 mmol) and stirring was continued at room temperature for 12 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue dissolved in ethyl acetate (200 mL) whereupon washing was performed with brine (2×100 mL). The separated organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [3–5% (v/v) MeOH in CH_2Cl_2] to give furanose **15** (4.20 g, 80%) as a clear oil. δ_C ($CDCl_3$) 137.1, 128.5, 128.4, 128.0, 127.9, 127.7, 112.9, 105.5, 85.5, 85.2, 82.7, 71.8, 62.7, 27.1, 26.3; MALDI-MS: *m*/*z* 303.1207 ($[M+Na]^+$, $C_{15}H_{20}O_5Na^+$ calcd 303.1208). The ^1H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[25]

7.1.15. 5-*O*-Benzoyl-3-*O*-benzyl-1,2-*O*-isopropylidene-β-L-arabinofuranose (16). Benzoyl chloride (1.6 mL, 13.9 mmol) was added dropwise to a solution of furanose **15** (2.60 g, 9.29 mmol) in anhydrous pyridine (10 mL) and the resulting mixture was stirred at room temperature for 2 h. The mixture was then concentrated to dryness under reduced pressure, the residue obtained was dissolved in ethyl acetate (100 mL), and washing was performed first with saturated aq $NaHCO_3$ (100 mL) and then with brine (100 mL). The separated organic phase was dried (Na_2SO_4), filtered, evaporated to dryness under reduced pressure and then coevaporated with toluene (2×5 mL). The residue obtained was purified by column chromatography [15–20% (v/v) EtOAc in light petroleum] to give furanose **16** (2.60 g, 74%) as a clear oil. δ_H ($CDCl_3$) 8.00 (2H, d, J=8.0 Hz), 7.59–7.25 (8H, m), 5.95 (1H, d, J=3.6 Hz, H1), 4.70 (1H, d, J=3.6 Hz, H2), 4.66 (1H, d, J=11.8 Hz, CH_2Ph), 4.58 (1H, d, J=11.9 Hz, CH_2Ph), 4.50–4.40 (3H, m, H4 and H5), 4.08 (1H, d, J=2.8 Hz, H3), 1.55 and 1.35 [3H each, 2s, CH_3(isopropylidene)]; δ_C ($CDCl_3$) 166.1, 136.9, 133.1, 129.7, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 113.1, 105.8, 84.8, 82.7, 82.2, 71.8, 64.3, 27.1, 26.3; MALDI-HRMS: *m*/*z* 407.1475 ($[M+Na]^+$, $C_{22}H_{24}O_6Na^+$ calcd 407.1471).

7.1.16. 1,2-Di-*O*-acetyl-5-*O*-benzoyl-3-*O*-benzyl-α,β-L-arabinofuranose (17). Concentrated sulfuric acid (0.02 mL) was added to a solution of furanose **16** (2.21 g, 5.76 mmol) in 80% aq acetic acid (20 mL) and stirring was continued for 1 h at 50 °C. The reaction mixture was allowed to cool to room temperature and then concentrated under reduced pressure to approximately half of the original volume. Pyridine (30 mL) and acetic anhydride (1.63 mL, 17.3 mmol) were added and the resulting mixture was stirred at 50 °C for 6 h and then concentrated to dryness under reduced pressure. The residue obtained was dissolved in ethyl acetate (50 mL) and then washed first with saturated aq $NaHCO_3$ (50 mL) followed by brine (50 mL). The separated organic phase was dried (Na_2SO_4), filtered, evaporated to dryness under reduced pressure and then coevaporated with toluene (2×5 mL). The residue obtained was purified by column chromatography [15–20% (v/v) EtOAc in light petroleum] yielding a 1:1 mixture of anomers **17** as a clear oil (1.80 g, 73%). δ_H ($CDCl_3$) 8.06–8.01 (4H, m), 7.60–7.55 (2H, m), 7.46–7.40 (4H, m), 7.30–7.26 (10H, m), 6.39 (1H, d, J=4.6 Hz), 6.23 (1H, s), 5.31 (1H, m), 5.27 (1H, s), 4.77 (1H, d, J=12.0 Hz), 4.70–4.34 (9H, m), 4.01–3.99 (2H, m), 2.12 (3H, s), 2.06 (3H, s), 2.05 (3H, s), 1.95 (3H, s), δ_C ($CDCl_3$) 169.6, 169.5, 169.2, 166.1, 166.0, 137.2, 137.1, 133.2, 133.1, 129.8, 129.7, 129.6, 129.5, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 99.9, 93.9, 83.1, 82.9, 80.6, 79.6, 79.5, 77.0, 72.7, 72.4, 64.3, 63.4, 21.1, 20.9, 20.7, 20.4; MALDI-HRMS: *m*/*z* 451.1375 ($[M+Na]^+$, $C_{23}H_{24}O_8Na^+$ calcd 451.1369).

7.1.17. 9-(2-*O*-Acetyl-5-*O*-benzoyl-3-*O*-benzyl-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (18). To a suspension of anomers **17** (0.70 g, 1.64 mmol) and 6-*N*-benzoyladenine (0.59 g, 2.45 mmol) in anhydrous acetonitrile (6 mL) was added $SnCl_4$ (0.4 mL, 3.3 mmol) and the resulting mixture was stirred at room temperature for 4 h. Saturated aq $NaHCO_3$ was added until the evolution of carbon dioxide ceased whereupon the mixture was filtered through a layer of Celite 545, that was subsequently flushed with $CHCl_3$ (2×50 mL). The combined filtrate was washed successively with saturated aq $NaHCO_3$ (3×100 mL) and brine (2×100 mL), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [4–5% (v/v) MeOH in

$CHCl_3$] affording nucleoside **18** (870 mg, 88%) as a white solid material. δ_H ($CDCl_3$) 9.16 (1H, br s, NH), 8.81 (1H, s, H8), 8.39 (1H, s, H2), 8.04–8.00 (4H, m), 7.62–7.42 (6H, m), 7.28–7.23 (5H, m), 6.48 (1H, s, H1′), 5.81 (1H, t, J=1.4 Hz, H2′), 4.83 (1H, m, H4′), 4.73 (1H, d, J=11.9 Hz, CH_2Ph), 4.65 (1H, d, J=12.0 Hz, CH_2Ph), 4.51 (1H, d, J=5.4 Hz, H5′a), 4.50 (1H, d, J=5.6 Hz, H5′b), 4.24 (1H, dd, J=1.3, 3.3 Hz, H3′), 2.11 (3H, s, $COCH_3$); δ_C ($CDCl_3$) 169.7, 166.2, 164.7, 153.0, 151.8, 149.7, 141.6, 136.4, 133.7, 133.5, 132.9, 129.9, 129.5, 129.0, 128.7, 128.6, 128.4, 128.04, 128.01, 123.1, 88.5, 84.3, 82.7, 80.5, 72.7, 63.5, 20.9; MALDI-HRMS: m/z 630.1966 ([M+Na]$^+$, $C_{33}H_{29}N_5O_7Na^+$ calcd 630.1965).

7.1.18. 6-*N*-Benzoyl-9-(5-*O*-benzoyl-3-*O*-benzyl-α-L-arabinofuranosyl)adenine (19). To a solution of nucleoside **18** (0.64 g, 1.06 mmol) in MeOH (16 mL) was added saturated methanolic ammonia (16 mL) and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was coevaporated with toluene (5×10 mL). The residue obtained was purified by column chromatography [5–6% (v/v) MeOH in $CHCl_3$] affording nucleoside **19** (550 mg, 92%) as a white solid material. δ_H ($CDCl_3$) 9.07 (1H, br s, NH), 8.71 (1H, s, H8), 8.21 (1H, s, H2), 8.05–8.01 (4H, m), 7.62–7.43 (6H, m), 7.30–7.25 (5H, m), 6.12 (1H, d, J=4.0 Hz, H1′), 4.97 (1H, t, J=4.7 Hz, H2′), 4.80 (1H, d, J=11.9 Hz, CH_2Ph), 4.75 (1H, m, H4′), 4.71 (1H, d, J=12.0 Hz, CH_2Ph), 4.63 (1H, dd, J=3.7, 12.4 Hz, H5′a), 4.51 (1H, dd, J=5.0, 12.3 Hz, H5′b), 4.33 (1H, t, J=5.3 Hz, H3′); δ_C ($CDCl_3$) 166.4, 164.7, 152.5, 151.1, 149.6, 141.4, 137.2, 133.6, 133.5, 133.1, 129.9, 129.8, 129.7, 129.1, 128.7, 128.6, 128.5, 128.3, 128.1, 128.0, 123.2, 91.4, 82.3, 80.6, 72.7, 63.9; MALDI-HRMS: m/z 588.1863 ([M+Na]$^+$, $C_{31}H_{27}N_5O_6Na^+$ calcd 588.1859).

7.1.19. 9-(2-*O*-Acetyl-5-*O*-benzoyl-3-*O*-benzyl-α-L-ribofuranosyl)-6-*N*-benzoyladenine (20). Nucleoside **19** (0.45 g, 0.80 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 (20 mL) and anhydrous pyridine (4 mL). The stirred solution was cooled to −30 °C and trifluoromethanesulfonic anhydride (0.35 mL, 2.15 mmol) was added. After 1.5 h, the reaction mixture was allowed to warm to 0 °C and saturated aq $NaHCO_3$ (10 mL) and CH_2Cl_2 (60 mL) were added. The organic phase was separated, washed with saturated aq $NaHCO_3$ (3×70 mL), dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was dissolved in a mixture of anhydrous toluene (24 mL) and anhydrous CH_2Cl_2 (24 mL) whereupon KOAc (0.39 g, 3.98 mmol) and 18-crown-6 (0.74 g, 2.79 mmol) were added at room temperature under stirring. The temperature was raised to 50 °C and stirring was continued for another 16 h. After cooling to room temperature, CH_2Cl_2 (100 mL) was added, and the reaction mixture was washed with saturated aq $NaHCO_3$ (3×50 mL), dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [3–5% (v/v) MeOH in $CHCl_3$] to yield nucleoside **20** (380 mg, 79%) as a white solid material. δ_H ($CDCl_3$) 9.17 (1H, br s, NH), 8.80 (1H, s, H8), 8.35 (1H, s, H2), 8.04–7.43 (15H, m), 6.47 (1H, d, J=5.2 Hz, H1′), 5.87 (1H, m, H2′), 4.72–4.25 (6H, m, H3′, H4′, H5′ and CH_2Ph), 2.08 (3H, s, $COCH_3$); δ_C ($CDCl_3$) 169.3, 166.2, 164.9, 152.8, 149.8, 149.6, 142.8, 136.5, 133.6, 132.9, 129.8, 129.4, 129.1, 128.9, 128.8, 128.7, 128.6, 128.3, 128.2, 128.1, 125.4, 123.9, 82.7, 81.0, 76.8, 73.8, 70.9, 63.5, 20.6; MALDI-HRMS: m/z 630.1960 ([M+Na]$^+$, $C_{33}H_{29}N_5O_7Na^+$ calcd 630.1965).

7.1.20. 6-*N*-Benzoyl-9-(α-L-ribofuranosyl)adenine (22). Aqueous sodium hydroxide (1, 2.7 mL) was added to an ice-cold solution of nucleoside **20** (0.36 g, 0.59 mmol) in a mixture of ethanol (1.8 mL) and pyridine (3.5 mL). The reaction mixture stirred at 0 °C for 30 min and then neutralized with Dowex 50WX2(H^+). Dowex was filtered off and the filtrate was concentrated to dryness under reduced pressure. The oily residue was dissolved in EtOAc (100 mL) whereupon washing was performed using brine (2×75 mL). The separated organic phase was dried (Na_2SO_4), filtrated and concentrated to dryness under reduced pressure. The residue obtained was coevaporated with toluene and purified by column chromatography [3–5% MeOH in EtOAc] to give nucleoside **21** (tentatively assigned) as a white solid material (0.21 g). Ammonium formate (200 mg) and Pd/C (100 mg) were added to a solution of nucleoside **21** (0.21 g) in EtOH (5 mL). The resulting mixture was heated under reflux for 2 h, cooled to room temperature, filtered across a pad of Celite and then concentrated to dryness. The crude product was recrystallized from EtOH to give nucleoside **22** as white needles (0.12 g, 54% from **20**). δ_C (DMSO-d_6) 165.5, 152.6, 151.4, 149.9, 144.9, 133.4, 132.4, 128.5, 125.0, 85.3, 83.6, 70.7, 70.6, 61.4; MALDI-HRMS: m/z 394.1120 ([M+Na]$^+$, $C_{17}H_{17}N_5O_5Na^+$ calcd 394.1127). The ^{1}H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.21. 6-*N*-Benzoyl-9-[5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl]adenine (23). 4,4′-Dimethoxytrityl chloride (0.33 g, 1.0 mmol) was added to a solution of nucleoside **22** (0.13 g, 0.35 mmol) in anhydrous pyridine (7 mL) and stirring was continued at room temperature for 12 h. MeOH (5 mL) was added and after stirring for another 10 min the reaction mixture was poured into saturated aq $NaHCO_3$ (25 mL). Extraction was performed with $CHCl_3$ (3×20 mL) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [6–8% (v/v) MeOH in $CHCl_3$] affording nucleoside **23** (220 mg, 92%) as a white foam. δ_C ($CDCl_3$) 158.7, 152.0, 151.1, 149.4, 144.7, 143.9, 143.8, 135.9, 135.7, 133.7, 133.0, 130.2, 130.0, 129.0, 128.2, 128.1, 128.0, 127.9, 127.8, 127.1, 122.9, 113.4, 113.2, 87.2, 86.9, 86.0, 72.8, 72.2, 64.4, 55.4.; ESI-HRMS: m/z 696.2429 ([M+Na]$^+$, $C_{38}H_{35}N_5O_7Na^+$ calcd 696.2434). The ^{1}H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.22. 6-*N*-Benzoyl-9-[2-*O*-*tert*-butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl]adenine (24). *tert*-Butyldimethylsilyl chloride (0.06 g, 0.43 mmol) was added to a solution of nucleoside **23** (0.22 g, 0.33 mmol) and imidazole (0.06 g, 0.85 mmol) in anhydrous pyridine (5 mL) and stirring was continued at room temperature for 15 h. The reaction mixture was poured into saturated aq $NaHCO_3$ (25 mL) and extraction was performed with

$CHCl_3$ (3×20 mL). The combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [4–5% (v/v) acetone in CH_2Cl_2] to give the required 2′-*O*-*tert*-butyldimethylsilyl isomer **24** as a white foam (0.12 g, 48%). Further elution [5–7% (v/v) acetone in CH_2Cl_2] yielded a byproduct tentatively assigned as the 3′-*O*-*tert*-butyldimethylsilyl isomer (yield not determined). δ_C ($CDCl_3$) 164.6, 158.73, 158.71, 152.5, 151.8, 149.5, 144.6, 143.6, 135.7, 135.4, 133.9, 132.8, 130.1, 130.0, 128.9, 128.1, 127.9, 127.1, 122.6, 113.4, 113.37, 86.9, 85.8, 85.4, 73.2, 72.9, 64.4, 55.3, 25.4, 17.8, −5.2, −5.3; ESI-HRMS: *m/z* 810.3286 ($[M+Na]^+$, $C_{44}H_{49}N_5O_7SiNa^+$ calcd 810.3299). The ^1H NMR data were found to be consistent with the literature data for the corresponding D-enantiomer.[12b]

7.1.23. 6-*N*-Benzoyl-9-[2-*O*-*tert*-butyldimethylsilyl-3-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5-*O*-(4,4′-dimethoxytrityl)-α-L-ribofuranosyl]adenine (25). To a stirred solution of nucleoside **24** (70 mg, 0.09 mmol) and *N*,*N*-diisopropylethylamine (0.16 mL, 0.92 mmol) in CH_2Cl_2 (2 mL) at room temperature was added 2-cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (0.09 mL, 0.46 mmol) and stirring was continued for 15 h. CH_2Cl_2 (10 mL) was added and the resulting mixture was washed with saturated aq $NaHCO_3$ (10 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [50–55% EtOAc in *n*-hexane, containing 0.5% Et_3N (v/v/v)] furnishing phosphoramidite **25** (65 mg, 73%) as a white foam. δ_P (DMSO-d_6) 151.8, 150.4; ESI-MS *m/z* 988.4 $[M+H]^+$, 1010.5 ($[M+Na]^+$, $C_{53}H_{66}N_7O_8PSiNa^+$ calcd 1010.5).

7.1.24. 1-[3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-hydroxymethyl-β-D-xylofuranosyl]thymine (27). A solution of nucleoside **26**[28] (5.50 g, 6.04 mmol) in saturated methanolic ammonia (100 mL) was stirred for 48 h at room temperature. After evaporation to dryness under reduced pressure, the resulting residue was coevaporated with toluene (2× 5 mL) and purified by column chromatography [60–66% (v/v) EtOAc in light petroleum] to afford nucleoside **27** (4.45 g, 96%) as a white solid material. R_f 0.24 (MeOH/CH_2Cl_2 15:85, v/v); δ_H ($CDCl_3$) 9.50 (1H, s), 7.66 (2H, dd, J=1.3, 7.9 Hz), 7.59 (2H, dd, J=1.3, 7.9 Hz), 7.50–7.31 (13H, m), 7.27–7.23 (4H, m), 5.86 (1H, d, J=2.9 Hz, H1′), 4.43 (1H, br s, 2′-OH), 4.41 (1H, d, J=2.7 Hz, H2′), 4.22 (1H, br s, H3′), 4.02 (1H, d, J=11.6 Hz, H5′a), 3.86 (1H, dd, J=4.5, 11.9 Hz, H5″a), 3.71 (1H, d, J=11.9 Hz, H5′b), 3.48 (1H, dd, J=6.0, 11.7 Hz, H5″b), 2.76 (1H, m, 5′-OH), 1.66 (3H, d, J=1.0 Hz, 5-CH_3), 1.06 and 0.91 (2× Si*C*(CH_3)$_3$); δ_C ($CDCl_3$) 164.1, 151.0, 136.0, 135.9, 135.8, 135.7, 132.9, 132.8, 132.6, 132.2, 130.3, 130.2, 130.0, 128.0, 127.9, 127.8, 110.8, 92.0, 90.7, 83.2, 79.6, 65.2, 63.6, 27.0, 26.9, 19.4, 19.2, 12.4; MALDI-MS: *m/z* 787 ($[M+Na]^+$, $C_{43}H_{52}N_2O_7Si_2Na^+$ calcd 787).

7.1.25. 1-[3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-β-D-xylofuranosyl]thymine (28). 4,4′-Dimethoxytrityl chloride (2.05 g, 6.05 mmol) was added in one portion to a stirred solution of nucleoside **27** (4.2 g, 5.49 mmol) in anhydrous pyridine (20 mL). After stirring the mixture 12 h at room temperature, toluene (20 mL) was added and the solution was concentrated to approximately one-fourth the original volume under reduced pressure. CH_2Cl_2 (100 mL) was added whereupon washing was performed with saturated aq $NaHCO_3$ (2× 50 mL). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was coevaporated with toluene (2×10 mL) and then purified by column hromatography [40–50% EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)] to afford nucleoside **28** as a white solid (5.68 g, 97%). R_f 0.33 (MeOH/CH_2Cl_2 5:95, v/v); δ_H ($CDCl_3$) 8.89 (1H, s, NH), 7.64–7.61 (2H, m), 7.58–7.55 (2H, m), 7.48 (1H, d, J= 1.2 Hz, H6), 7.44–7.40 (7H, m), 7.36–7.30 (9H, m), 7.28–7.12 (9H, m), 6.73 (4H, d, J=8.7 Hz), 5.82 (1H, d, J= 3.7 Hz, H1′), 4.35 (1H, d, J=3.2 Hz, H3′), 4.07 (1H, m, H2′), 4.03 (1H, d, J=11.7 Hz, H5′a), 3.92 (1H, d, J= 11.8 Hz, H5′b), 3.77 and 3.76 (3H each, 2s, 2×OCH_3), 3.57 (1H, d, J=9.6 Hz, H5″a), 3.18 (1H, d, J=9.5 Hz, H5″b), 2.89 (1H, d, J=5.4 Hz, 2′-OH), 1.65 (3H, s, 5-CH_3), 1.00 and 0.83 (9H each, 2s, 2×C(CH_3)$_3$); δ_C ($CDCl_3$) 163.9 (C4), 158.5, 158.4, 150.8 (C2), 144.4, 136.0, 135.8, 135.7, 135.6, 135.5, 133.2, 133.0, 132.9, 132.0, 130.4, 130.2, 130.1, 129.9, 129.8, 128.4, 127.9, 127.8, 126.8, 113.2, 110.5 (C5), 91.4 (C1′), 89.9 (C4′), 87.0 (CAr_3), 83.2 (C2′), 80.0 (C3′), 65.0 and 63.6 (C5′ and C5″), 55.3 (2×OCH_3), 27.0 and 26.9 (2×C(*C*H_3)$_3$), 19.5 and 19.2 (2×Si*C*(CH_3)$_3$), 12.4 (5-CH_3); MALDI-HRMS: *m/z* 1089.4576 ($[M+Na]^+$, $C_{64}H_{70}N_2O_9Si_2Na^+$ calcd 1089.4512).

7.1.26. 1-[3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-2-*O*-methanesulfonyl-β-D-xylofuranosyl]thymine (29) and 2,2′-anhydro-1-[3,5-di-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-β-D-lyxofuranosyl]thymine (30). Nucleoside **28** (3.2 g, 3.0 mmol) was dissolved in a 1:1 mixture of anhydrous CH_2Cl_2–Et_3N (10 mL). DMAP (440 mg, 3.6 mmol) was added followed by methanesulfonyl chloride (413 mg, 3.6 mmol) and the resulting mixture was stirred at room temperature for 12 h. Analytical TLC showed the formation of two products. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washing was performed with saturated aq $NaHCO_3$ (2×50 mL). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. An analytical sample was purified by column chromatography [40–45% (v/v) EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)] to give as the major product nucleoside **29**, R_f 0.38 (MeOH/CH_2Cl_2 5:95, v/v), and [70–75% (v/v) EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)] as the minor product the anhydro nucleoside **30**, R_f 0.28 (MeOH/CH_2Cl_2 5:95, v/v), (both as white solid materials). Data for compound **29**: δ_H ($CDCl_3$) 8.70 (1H, s, NH), 7.70–7.68 (4H, m), 7.54–7.52 (2H, m), 7.47–7.25 (16H, m), 7.23–7.14 (8H, m), 6.76–6.73 (4H, m), 6.03 (1H, d, J=4.7 Hz, H1′), 5.17 (1H, dd, J=4.3, 4.7 Hz, H2′), 4.67 (1H, d, J=4.1 Hz, H3′), 4.17 (1H, d, J= 11.2 Hz, H5′a), 4.12 (1H, d, J=11.2 Hz, H5′b), 3.77 and 3.76 (3H each, 2s, 2×OCH_3), 3.39 (1H, d, J=9.4 Hz, H5″a), 3.09 (1H, d, J=9.1 Hz, H5″b), 2.50 (3H, s, SO_2CH_3), 1.44 (3H, s, 5-CH_3), 1.03 and 0.87 (9H each, 2s, 2×C(CH_3)$_3$); δ_C ($CDCl_3$) 163.5 (C4), 158.5, 150.4 (C2), 144.7, 136.0, 135.9, 135.8, 135.6, 135.4, 133.5, 132.9, 132.3, 131.1, 130.5, 130.3, 130.2, 129.9, 128.3, 128.1,

128.0, 127.9, 126.8, 113.2, 111.8 (C5), 88.3 (C4′), 86.7 (CAr_3), 85.6 (C1′), 85.2 (C2′), 77.0 (C3′), 64.4 (C5′), 62.5 (C5″), 55.3 (2×OCH_3), 38.3 (SO_2CH_3), 27.2 and 27.0 (2×$C(CH_3)_3$), 19.6 and 19.3 (2×$SiC(CH_3)_3$), 11.9 (5-CH_3); data for compound **30**: δ_H ($CDCl_3$) 7.57–7.54 (2H, m), 7.41–7.19 (22H, m), 7.16–7.07 (6H, m), 6.77–6.73 (4H, m), 6.07 (1H, d, J=6.2 Hz, H1′), 4.82 (1H, dd, J=6.0, 6.3 Hz, H2′), 4.65 (1H, d, J=5.9 Hz, H3′), 3.87 (1H, d, J=10.5 Hz, H5′a), 3.79 (6H, s, 2×OCH_3), 3.58 (1H, d, J=12.2 Hz, H5″a), 3.15 (1H, d, J=12.2 Hz, H5″b), 2.75 (1H, d, J=10.3 Hz, H5′b), 1.99 (3H, s, 5-CH_3), 0.88 and 0.81 (9H each, 2s, 2×$C(CH_3)_3$); δ_C ($CDCl_3$) 172.4 (C4), 159.9 (C2), 158.6, 144.5, 136.1, 135.8, 135.6, 135.5, 135.4, 133.1, 132.7, 132.5, 132.3, 130.3, 130.2, 130.1, 129.9, 129.8, 129.7, 128.1, 127.9, 127.8, 127.7, 127.6, 127.0, 119.0 (C5), 113.2, 90.8 (C4′), 89.3 (C1′), 86.9 (CAr_3), 81.2 (C2′), 73.3 (C3′), 64.9 and 64.4 (C5′ and C5″), 55.3 (2×OCH_3), 26.8 and 26.7 (2×$C(CH_3)_3$), 19.4 and 19.2 (2×$SiC(CH_3)_3$), 14.2 (5-CH_3); MALDI-HRMS: m/z 1071.4466 ($[M+Na]^+$, $C_{64}H_{68}N_2O_8Si_2Na^+$ calcd 1071.4406).

7.1.27. 1-[5-*O*-(4,4′-Dimethoxytrityloxymethyl)-4-*C*-hydroxymethyl-α-L-ribofuranosyl]thymine (31). The crude mixture (3.35 g) obtained from the mesylation of **28** was dissolved in ethanol (50 mL), H_2O (45 mL) and aq NaOH (2 M solution, 5.0 mL) were added and the resulting solution was heated under reflux for 16 h. Toluene (100 mL) was added and the resulting mixture was concentrated to approximately one-third of the original volume. After partitioning between EtOAc (200 mL) and saturated aq $NaHCO_3$ (200 mL), the aqueous phase was separated and extracted with EtOAc (100 mL). The combined organic phase was washed with brine (200 mL), dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [4–6% MeOH in CH_2Cl_2, containing 0.5% Et_3N (v/v/v)] to afford nucleoside **31** as a white solid material (1.15 g, 65% from **28**). R_f 0.12 (MeOH/CH_2Cl_2 5:95, v/v); δ_H ($CDCl_3$) 10.30 (1H, s, NH), 7.81 (1H, s, H6), 7.40 (2H, d, J=7.3 Hz), 7.31–7.15 (7H, m), 6.81 (4H, d, J=8.5 Hz), 6.09 (1H, d, J=3.8 Hz, H1′), 4.66 (1H, dd, J=4.3, 4.6 Hz, H2′), 4.38 (1H, d, J=5.1 Hz, H3′), 3.90 (2H, br s, H5″), 3.74 (6H, s, 2×OCH_3), 3.25 (1H, d, J=9.9 Hz, H5′a), 3.17 (1H, d, J=10.1 Hz, H5′b), 1.81 (3H, s, 5-CH_3); δ_C ($CDCl_3$) 165.4, 158.6, 151.0, 144.5, 138.5, 135.6, 135.5, 130.2, 130.1, 128.1, 128.0, 127.0, 113.3, 113.2, 108.9, 87.3, 86.8, 86.2, 73.9, 70.9, 66.2, 63.3, 55.3, 12.5; MALDI-HRMS: m/z 613.2180 ($[M+Na]^+$, $C_{32}H_{34}N_2O_9Na^+$ calcd 613.2157).

7.1.28. 1-[2,5-Di-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-β-D-lyxofuranosyl]thymine (32) and 1-[3,5-di-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-β-D-lyxofuranosyl]thymine (33). *tert*-Butyldimethylsilyl chloride (1.21 g, 8.0 mmol) and imidazole (1.09 g, 16.0 mmol) were added to a stirred solution of nucleoside **31** (1.18 g, 2.0 mmol) in anhydrous pyridine (10 mL). The reaction mixture was stirred at room temperature for 12 h and MeOH (1.0 mL) was then added. After stirring for 30 min the reaction mixture was concentrated to dryness under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with saturated aq $NaHCO_3$ (2×50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness. The residue was coevaporated with toluene (2×5.0 mL) and purified by column chromatography [30–40% (v/v) EtOAc in light petroleum] to give nucleoside **32** (955 mg, 58%) and [45–50% (v/v) EtOAc in light petroleum] nucleoside **33** (384 mg, 23%) (both as white solid materials). R_f 0.26, 0.32 (MeOH/CH_2Cl_2 5:95, v/v); data for **32**: δ_H ($CDCl_3$) 8.74 (1H, s, NH), 7.43–7.39 (3H, m), 7.31–7.19 (7H, m), 6.82 (4H, d, J=8.7 Hz), 6.48 (1H, d, J=5.9 Hz, H1′), 4.77 (1H, dd, J=5.3, 5.5 Hz, H2′), 4.04–4.00 (2H, m, H3′ and H5′a), 3.77–3.74 (7H, m, H5′b and 2×OCH_3), 3.33 (1H, d, J=9.8 Hz, H5″a), 3.23 (1H, d, J=10.1 Hz, H5″b), 2.89 (1H, d, J=1.6 Hz, 3′-OH), 1.91 (3H, s, 5-CH_3), 0.80 and 0.76 (9H each, 2s, 2×$C(CH_3)_3$), 0.04, −0.02, −0.04 and −0.06 (3H each, 4s, 4×$SiCH_3$); δ_c ($CDCl_3$) 163.8 (C4), 158.6, 150.7 (C2), 144.5, 138.2, 135.8, 135.5, 130.1, 130.0, 128.1, 128.0, 127.0, 113.4, 113.3, 109.2 (C5), 87.2, 86.8 and 85.2 (C1′, C4′ and CAr_3), 73.0 and 72.7 (C2′ and C3′), 66.1 and 63.5 (C5′ and C5′), 55.3 (2×OCH_3), 25.8 and 25.6 (2×$C(CH_3)_3$), 18.2 and 18.0 (2×$C(CH_3)_3$), 12.6 (5-CH_3), −5.2, −5.3, −5.4 and −5.5 (4×$SiCH_3$); MALDI-HRMS: m/z 841.3900 ($[M+Na]^+$, $C_{44}H_{62}N_2O_9Si_2Na^+$ calcd 841.3886); data for **33**: δ_H ($CDCl_3$) 8.81 (1H, s, NH), 7.60 (1H, d, J=1.0 Hz, H6), 7.42 (2H, d, J=7.2 Hz), 7.33–7.21 (7H, m), 6.83 (4H, d, J=9.0 Hz), 6.10 (1H, d, J=2.7 Hz, H1′), 4.67 (1H, d, J=10.2 Hz, 2′-OH), 4.53 (1H, d, J=4.8 Hz, H3′), 4.12 (1H, ddd, J=2.7, 5.1, 10.2 Hz, H2′), 3.79 (6H, s, 2×OCH_3), 3.74 (1H, d, J=10.6 Hz, H5′a), 3.59 (1H, d, J=10.8 Hz, H5′b), 3.15 (1H, d, J=9.9 Hz, H5″a), 3.04 (1H, d, J=9.9 Hz, H5″b), 1.94 (3H, s, 5-CH_3), 0.95 and 0.81 (9H each, 2s, 2×$C(CH_3)_3$), 0.16, 0.14, 0.02 and −0.05 (3H each, 4s, 4×$SiCH_3$); δ_C ($CDCl_3$) 163.9, 158.6, 150.4, 144.4, 137.5, 135.6, 135.5, 130.1, 130.0, 128.1, 128.0, 127.0, 113.3, 108.8, 86.9, 86.6, 85.0, 74.4, 71.2, 65.3, 63.5, 55.3, 26.0, 25.8, 18.3, 18.1, 12.6, −4.7, −5.1, −5.4 and −5.5; MALDI-HRMS: m/z 841.3908 ($[M+Na]^+$, $C_{44}H_{62}N_2O_9Si_2Na^+$ calcd 841.3886).

7.1.29. 1-[3-*O*-(2-Cyanoethoxy(*N*,*N*-diisopropylamino)-phosphino)-2,5-di-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-β-D-lyxofuranosyl]thymine (34). 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (473 mg, 2.0 mmol) was added dropwise to a stirred solution of the nucleoside **32** (819 mg, 1.0 mmol) and *N*,*N*-diisopropylethylamine (1.0 mL) in anhydrous CH_2Cl_2 (10 mL). After stirring the resulting mixture for 12 h at room temperature, the reaction mixture was diluted with EtOAc (50 mL). Washing was performed with saturated aq $NaHCO_3$ (2×25 mL). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [33–40% EtOAc in *n*-hexane containing 0.5% Et_3N (v/v/v)] to give amidite **34** as a white solid material (723 mg, 71%). R_f 0.31, 0.32 (MeOH/CH_2Cl_2 5:95, v/v); δ_P 153.9 and 151.6; δ_H ($CDCl_3$, major isomer) 8.13 (br s, NH), 7.52 (s, H6), 7.42 (d, J=7.7 Hz), 7.32 (d, J=8.8 Hz), 7.27–7.21 (m), 6.84 (d, J=8.7 Hz), 6.35 (d, J=5.7 Hz, H1′), 4.59 (dd, J=5.1, 5.7 Hz, H2′), 4.45 (dd, J=5.1, 13.7 Hz, H3′), 4.02 (d, J=11.3 Hz, H5′a), 3.84 (d, J=11.6 Hz, H5′b), 3.79 (s, 2×OCH_3), 3.75–3.56 (m, OCH_2 and 2×$CH(CH_3)_2$), 3.44 (d, J=9.9 Hz, H5″a), 3.22 (d, J=9.8 Hz, H5″b), 2.34 (dd, J=6.5, 11.4 Hz, CH_2CN), 1.90 (s, 5-CH_3), 1.19 (d, J=6.3 Hz, $C(CH_3)_2$), 1.18 (d, J=6.4 Hz, $C(CH_3)_2$), 0.81 (s, 2×$C(CH_3)_3$), 0.06,

−0.05, −0.06 and −0.10 (4s, 4×$SiCH_3$); MALDI-HRMS: *m/z* 1041.4963 ($[M+Na]^+$, $C_{53}H_{79}N_4O_{10}PSi_2Na^+$ calcd 1041.4965).

7.1.30. 1-[2-*O*-Acetyl-5-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-α-L-arabinofuranosyl]thymine (36). *tert*-Butyldimethylsilyl chloride (2.0 g, 13.3 mmol), imidazole (1.82 g, 26.7 mmol) and DMAP (100 mg, 0.82 mmol) were added to a stirred solution of 1-[2-*O*-acetyl-5-*O*-benzoyl-4-*C*-(4,4′-dimethoxytrityloxymethyl)-α-L-arabinofuranosyl]thymine (**35**[28], 3.28 g, 4.45 mmol) dissolved in anhydrous DMF (10 mL). The reaction mixture was allowed to stir at 36 °C for 12 h and was then partitioned between CH_2Cl_2 (100 mL) and saturated aq $KHSO_4$ (100 mL). The separated organic phase was washed with saturated aq $NaHCO_3$ (50 mL), then dried (Na_2SO_4), concentrated and coevaporated with toluene (3×5.0 mL). The crude product was purified by column chromatography [45–50% (v/v) EtOAc in light petroleum] to give nucleoside **36** as white solid material (3.33 g). R_f 0.2 (MeOH/CH_2Cl_2 5:95, v/v); δ_H ($CDCl_3$) 8.79 (1H, s, NH), 7.83 (2H, d, *J*=7.8 Hz), 7.58 (1H, dd, *J*=7.4, 7.6 Hz), 7.47–7.39 (6H, m), 7.34 (4H, d, *J*=8.5 Hz), 7.28–7.21 (2H, m), 6.78 (4H, d, *J*=8.7 Hz), 6.30 (1H, d, *J*=3.5 Hz, H1′), 5.26 (1H, dd, *J*=3.3, 3.7 Hz, H2′), 4.65 (1H, d, *J*=10.8 Hz, H5″a), 4.56 (1H, d, *J*=10.9 Hz, H5″b), 4.28 (1H, d, *J*=3.2 Hz, H3′), 3.76 (6H, s, 2×OCH_3), 3.64 (1H, d, *J*=10.2 Hz, H5′a), 3.35 (1H, d, *J*=10.1 Hz, H5′b), 2.13 (3H, s, $COCH_3$), 1.57 (3H, s, 5-CH_3), 0.70 (9H, s, $C(CH_3)_3$), −0.01 (3H, s, $SiCH_3$), −0.19 (3H, s, $SiCH_3$); δ_C ($CDCl_3$) 169.7, 165.7, 163.6, 158.7, 158.6, 150.4, 144.3, 136.0, 135.7, 135.3, 133.4, 130.3, 130.2, 129.8, 129.4, 128.5, 128.4, 128.0, 127.1, 113.3, 111.1, 88.1, 87.4, 86.8, 82.0, 76.3, 63.1, 55.3, 25.6, 20.9, 17.8, 12.2, −4.9, −5.5. NMR spectroscopic data revealed the compound to be contaminated with traces of DMF; MALDI-HRMS: *m/z* 873.3393 ($[M+Na]^+$, $C_{47}H_{54}N_2O_{11}SiNa^+$ calcd 873.3389).

7.1.31. 1-[5-*O*-Benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-α-L-arabinofuranosyl]-thymine (37). To a stirred solution of nucleoside **36** (3.0 g, 3.53 mmol) in MeOH (50 mL) was added methanol saturated with ammonia (10 mL) and the resulting mixture was stirred at room temperature for 5 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue obtained was coevaporated with toluene (2×2 mL). The crude product was purified by column chromatography [50–60% (v/v) EtOAc in light petroleum] furnishing nucleoside **37** as a white solid material (2.6 g, 80% from **35**). R_f 0.13 (MeOH/CH_2Cl_2 5:95, v/v); δ_H ($CDCl_3$) 10.50 (1H, s, NH), 7.82–7.80 (2H, m), 7.53–7.47 (5H, m), 7.45–7.31 (6H, m), 7.23–7.19 (2H, m), 6.75–6.71 (4H, m), 6.12 (1H, s, H1′), 5.41 (1H, d, *J*=3.5 Hz, 2′-OH), 4.92 (1H, d, *J*=10.3 Hz, H5′a), 4.74 (1H, d, *J*=10.8 Hz, H5′b), 4.32 (1H, br s, H3′), 4.17 (1H, br s, H2′), 3.73 and 3.72 (3H each, 2s, 2×OCH_3), 3.63 (1H, d, *J*=10.7 Hz, H5″a), 3.31 (1H, d, *J*=10.8 Hz, H5″b), 1.57 (3H, s, 5-CH_3), 0.66 (9H, s, $C(CH_3)_3$), −0.03 (3H, s, $SiCH_3$), −0.15 (3H, s, $SiCH_3$); δ_C ($CDCl_3$) 165.6 (COPh), 164.6 (C4), 158.6, 158.5, 151.0 (C2), 144.6, 136.5, 135.9, 135.5, 133.0, 130.2, 130.0, 129.9, 129.7, 128.3, 128.2, 128.0, 126.9, 113.3, 113.2, 110.1 (C5), 93.3 (C1′), 90.3 (C4′), 86.3 (CAr_3), 83.5 (C2′), 78.2 (C3′), 63.3 and 63.1 (C5′ and C5″), 55.2 (2×OCH_3), 25.4 ($C(CH_3)_3$), 17.7 ($C(CH_3)_3$), 12.5 (5-CH_3), −5.1 ($SiCH_3$), −5.6 ($SiCH_3$); MALDI-HRMS: *m/z* 831.3273 ($[M+Na]^+$, $C_{45}H_{52}N_2O_{10}SiNa^+$ calcd 831.3283).

7.1.32. 1-[5-*O*-Benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-2-*O*-methanesulfonyl)-α-L-arabinofuranosyl]thymine (38). Nucleoside **37** (2.56 g, 3.16 mmol) was dissolved in a 3:1 mixture of anhydrous CH_2Cl_2 and Et_3N (20 mL), and DMAP (580 mg, 4.75 mmol) was added. Methanesulfonyl chloride (544 mg, 4.75 mmol) was added dropwise and the reaction mixture was stirred at room temperature. After 4 h analytical TLC showed the formation of two products. Saturated aq $NaHCO_3$ (50 mL) was added and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2×50 mL) and the combined organic phase was washed first with aq HCl (1 M, 2×50 mL) and then with saturated aq $NaHCO_3$ (50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The crude product was coevaporated with toluene affording a white foam. An analytical sample was obtained by quick fractionation through silica gel [50–60% (v/v) EtOAc in light petroleum] affording the major product nucleoside **38** as a white solid material. R_f 0.23 (MeOH/CH_2Cl_2 5:95, v/v); δ_H ($CDCl_3$) 9.51 (1H, s, NH), 7.85–7.82 (2H, m), 7.58 (1H, m), 7.45–7.38 (6H, m), 7.33–7.29 (4H, m), 7.24–7.22 (2H, m), 6.78–6.74 (4H, m), 6.29 (1H, d, *J*=3.1 Hz, H1′), 5.08 (1H, dd, *J*=2.6, 2.8 Hz, H2′), 4.84 (1H, d, *J*=11.3 Hz, H5′a), 4.49 (1H, d, *J*=2.8 Hz, H3′), 4.41 (1H, d, *J*=10.9 Hz, H5′b), 3.75 and 3.74 (3H each, 2s, 2×OCH_3), 3.65 (1H, d, *J*=10.4 Hz, H5″a), 3.37 (1H, d, *J*=10.7 Hz, H5″b), 3.20 (3H, s, SO_2CH_3), 1.54 (3H, s, 5-CH_3), 0.69 (9H, s, $C(CH_3)_3$), −0.02 (3H, s, $SiCH_3$), −0.13 (3H, s, $SiCH_3$); δ_C ($CDCl_3$) 165.5 (COPh), 163.7 (C4), 158.7, 158.6, 150.7 (C2), 144.1, 135.5, 135.4, 135.1, 133.4, 130.3, 130.2, 130.1, 129.8, 129.4, 128.5, 128.4, 128.1, 127.1, 113.4, 113.3, 111.3 (C5), 89.3 (C4′), 88.1 (C1′), 86.8 and 86.6 (C2′ and CAr_3), 76.6 (C3′), 62.9 and 62.8 (C5′ and C5″), 55.3 (2×OCH_3), 39.0 (SO_2CH_3), 25.5 ($C(CH_3)_3$), 17.7 ($C(CH_3)_3$), 12.3 (5-CH_3), −4.8 ($SiCH_3$), −5.6 ($SiCH_3$); MALDI-HRMS: *m/z* 909.3040 ($[M+Na]^+$, $C_{46}H_{54}N_2O_{12}SSiNa^+$ calcd 909.3059).

7.1.33. 2,2′-Anhydro-1-[5-*O*-benzoyl-3-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-α-L-ribofuranosyl]thymine (39). The crude product obtained after mesylation of nucleoside **37** was coevaporated with anhydrous CH_3CN (2×5 mL) and then dissolved in CH_3CN (10 mL), and DBU (609 mg, 4 mmol) was added. The resulting mixture was stirred 12 h at room temperature and then evaporated to dryness under reduced pressure. $CHCl_3$ (50 mL) was added whereupon washing was performed with saturated aq $NaHCO_3$ (2×50 mL). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue was purified by column chromatography [4% (v/v) MeOH in CH_2Cl_2] to give nucleoside **39** as a white solid material (2.08 g, 83% from **37**). R_f 0.11 (MeOH/CH_2Cl_2 5:95, v/v); δ_H ($CDCl_3$) 7.91–7.89 (2H, m), 7.60 (1H, dd, *J*=7.3, 7.6 Hz), 7.44 (2H, dd, *J*=7.4, 7.9 Hz), 7.36–7.33 (2H, m), 7.27–7.12 (8H, m), 6.77–6.72 (4H, m), 6.15 (1H, d, *J*=5.8 Hz), 5.23 (1H, dd,

J=5.9, 6.0 Hz), 4.80 (1H, d, J=12.1 Hz), 4.49 (1H, d, J=6.2 Hz), 4.29 (1H, d, J=11.8 Hz), 3.76 (3H, s), 3.75 (3H, s), 3.34 (1H, d, J=11.3 Hz), 3.21 (1H, d, J=11.3 Hz), 1.97 (3H, s), 0.67 (9H, s), 0.04 (3H, s), −0.15 (3H, s); δ_C ($CDCl_3$) 172.1, 165.9, 159.9, 158.6, 144.5, 135.7, 135.2, 133.6, 130.2, 130.0, 129.9, 129.7, 129.4, 128.7, 128.0, 127.9, 127.0, 118.9, 113.3, 113.2, 89.2, 88.9, 86.3, 81.5, 73.5, 64.5, 63.3, 55.3, 25.6, 17.9, 14.3, −4.6, −5.6; MALDI-HRMS: m/z 813.3160 ($[M+Na]^+$, $C_{45}H_{50}N_2O_9SiNa^+$ calcd 813.3178).

7.1.34. 1-[5-*O*-(4,4′-Dimethoxytrityl)-4-*C*-hydroxymethyl-β-D-lyxofuranosyl]thymine (40). To a suspension of nucleoside **39** (1.7 g, 2.15 mmol) in a 1:1 mixture of EtOH and H_2O (20 mL) was added 2 M aqueous sodium hydroxide (1.5 mL), and the reaction mixture was heated under reflux for 6 h, then cooled and evaporated to approximately half of the original volume. The residue was partitioned between EtOAc (100 mL) and $NaHCO_3$ (50 mL). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The crude product was purified by column chromatography [6–7% (v/v) MeOH in CH_2Cl_2] to give nucleoside **40** as a white solid material (935 mg, 74%). R_f 0.21 (MeOH/CH_2Cl_2 10:90, v/v); δ_H ($CDCl_3$) 10.30 (1H, s), 7.49 (1H, s), 7.45 (2H, d, J=7.3 Hz), 7.33 (4H, d, J=7.7 Hz), 7.25–7.15 (3H, m), 6.79 (4H, d, J=8.3 Hz), 6.28 (1H, d, J=5.2 Hz), 5.17 (1H, d, J=4.9 Hz), 4.75 (1H, m), 4.32 (1H, dd, J=4.3, 4.4 Hz), 3.79 (1H, m), 3.74–3.62 (7H, m), 3.58 (1H, d, J=2.9 Hz), 3.53 (1H, d, J=10.3 Hz), 3.38 (1H, d, J=10.4 Hz), 3.34 (1H, br s), 1.64 (3H, s); δ_C ($CDCl_3$) 165.4, 158.6, 151.3, 144.6, 139.0, 135.7, 135.5, 130.2, 130.1, 128.2, 128.0, 127.0, 113.3, 109.0, 88.1, 86.8, 85.8, 72.5, 71.3, 65.5, 63.5, 55.3, 12.4.

7.1.35. 1-[2,5-Di-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-α-L-ribofuranosyl]thymine (41) and 1-[3,5-di-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-α-L-ribofuranosyl]thymine (42). *tert*-Butyldimethylsilyl chloride (816 mg, 5.42 mmol) and imidazole (740 mg, 10.9 mmol) were added to a stirred solution of nucleoside **40** (800 mg, 1.35 mmol) dissolved in anhydrous pyridine (8 mL). The reaction mixture was stirred at room temperature for 12 h whereupon MeOH (1.0 mL) was added. After stirring for 30 min the resulting mixture was concentrated to dryness under reduced pressure. The residue was dissolved in EtOAc (100 mL) and washed with saturated aq $NaHCO_3$ (2×50 mL). The organic phase was dried (Na_2SO_4), filtered, concentrated to dryness under reduced pressure and coevaporated with toluene (2×5 mL). The crude product was purified by column chromatography [30–35% (v/v) EtOAc in light petroleum] yielding nucleoside **41** (395 mg, 36%) and [35–45% (v/v) EtOAc in light petroleum] nucleoside **42** (590 mg, 53%), both as white solid materials. R_f 0.24, 0.34 (MeOH/CH_2Cl_2 5:95, v/v); data for **41**: δ_H ($CDCl_3$) 8.45 (1H, s, NH), 7.47–7.44 (2H, m), 7.33 (4H, dd, J=1.8, 8.9 Hz), 7.27–7.18 (4H, m), 6.80 (4H, dd, J=1.8, 8.9 Hz), 6.39 (1H, d, J=6.2 Hz, H1′), 4.76 (1H, dd, J=5.4, 6.1 Hz, H2′), 4.11 (1H, dd, J=2.2, 5.5 Hz, H3′), 3.87 (1H, d, J=10.7 Hz, H5′a), 3.76 (6H, s, 2×OCH_3), 3.70 (1H, d, J=10.8 Hz, H5′b), 3.49 (1H, d, J=9.9 Hz, H5″a), 3.33 (1H, d, J=10.1 Hz, H5″b), 2.67 (1H, br s, 3′-OH), 1.80 (3H, s, 5-CH_3), 0.91 and 0.78 (9H each, 2s, 2×$C(CH_3)_3$), 0.10 (3H, s, $SiCH_3$), 0.07 (6H, s, 2×$SiCH_3$), −0.01 (3H, s, $SiCH_3$); δ_C ($CDCl_3$) 163.8 (C4), 158.7, 158.6, 150.5 (C2), 144.8, 138.3, 136.0, 135.7, 130.3, 130.2, 128.3, 128.0, 126.9, 113.2, 109.1 (C5), 88.4 (C4′), 86.4 (CAr_3), 85.6 (C1′), 72.9 (C2′ and C3′), 67.3 (C5′), 63.7 (C5″), 55.3 (2×OCH_3), 26.1 and 25.6 (2×$C(CH_3)_3$), 18.4 and 18.0 (2×$C(CH_3)_3$), 12.6 (5-CH_3), −5.2, −5.3, −5.4 and −5.5 (4×$SiCH_3$); MALDI-HRMS: m/z 841.3859 ($[M+Na]^+$, $C_{44}H_{62}N_2O_9Si_2Na^+$ calcd 841.3886); data for **42**: δ_H ($CDCl_3$) 8.83 (1H, s, NH), 7.54 (1H, s, H6), 7.46–7.44 (2H, m), 7.36–7.22 (7H, m), 6.81 (4H, d, J=9.0 Hz), 6.23 (1H, d, J=4.1 Hz, H1′), 4.52 (1H, d, J=5.4 Hz, H3′), 4.39 (1H, m, H2′), 3.78 (6H, s, 2×OCH_3), 3.73 (1H, d, J=10.6 Hz, H5′a), 3.68 (1H, d, J=10.7 Hz, H5′b), 3.47 (1H, d, J=10.4 Hz, H5″a), 3.24 (1H, d, J=6.6 Hz, 2′-OH), 3.16 (1H, d, J=10.5 Hz, H5″b), 1.69 (3H, s, 5-CH_3), 0.87 and 0.76 (9H each, 2s, 2×$C(CH_3)_3$), 0.07, 0.03, 0.01 and −0.11 (3H each, 4s, 4×$SiCH_3$); δ_C ($CDCl_3$) 164.0 (C4), 158.7, 158.6, 150.7 (C2), 144.5, 138.0, 135.9, 135.6, 130.3, 130.2, 128.4, 128.0, 127.1, 113.3, 113.2, 109.1 (C5), 87.5 (C4′), 87.1 (CAr_3), 85.4 (C1′), 73.4 (C3′), 71.7 (C2′), 64.7 and 64.1 (C5′ and C5″), 55.3 (2×OCH_3), 25.9 and 25.7 (2×$C(CH_3)_3$), 18.3 and 18.2 (2×$C(CH_3)_3$), 12.6 (5-CH_3), −5.0, −5.2, −5.3 and −5.4 (4×$SiCH_3$); MALDI-HRMS: m/z 841.3891 ($[M+Na]^+$, $C_{44}H_{62}N_2O_9Si_2Na^+$ calcd 841.3886).

7.1.36. 1-[3-*O*-(2-Cyanoethoxy(diisopropylamino)-phosphino)-2,5-di-*O*-(*tert*-butyldimethylsilyl)-4-*C*-(4,4′-dimethoxytrityloxymethyl)-α-L-ribofuranosyl]thymine (43). Cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (170 mg, 0.72 mmol) was added dropwise to a stirred solution of nucleoside **41** (295 mg, 0.36 mmol) and *N*,*N*′-diisopropylethylamine (0.5 mL) in anhydrous CH_2Cl_2 (5 mL). After stirring for 12 h at room temperature, the reaction mixture was diluted with EtOAc (50 mL). Washing was performed with saturated aq $NaHCO_3$ (2×25 mL). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [30–35% EtOAc in *n*-hexane containing 0.5% Et_3N (v/v/v)] to give amidite **43** as a white solid material (242 mg, 66%). R_f 0.32, 0.35 (MeOH/CH_2Cl_2 5:95, v/v); δ_P 152.9 and 150.7; δ_H ($CDCl_3$, major isomer) 8.20 (br s, NH), 7.41 (d, J=8.1 Hz), 7.40 (s, H6), 7.32–7.16 (m), 6.78 (d, J=8.7 Hz), 6.20 (d, J=4.1 Hz, H1′), 4.69 (dd, J=4.8, 13.6 Hz, H3′), 4.40 (dd, J=4.3, 4.5 Hz, H2′), 4.22 (d, J=10.8 Hz, H5′a), 3.77 (s, 2×OCH_3), 3.74–3.68 (m, H5″a and OCH_2), 3.54 (d, J=10.8 Hz, H5′b), 3.44–3.41 (m, 2×$CH(CH_3)_2$), 3.17 (d, J=10.5 Hz, H5″b), 2.56 (t, J=6.4 Hz, CH_2CN), 1.78 (s, 5-CH_3), 1.11 (d, J=6.6 Hz, $C(CH_3)_2$), 0.93 (s, $C(CH_3)_3$), 0.87 (d, J=6.7 Hz, $C(CH_3)_2$), 0.71 (s, $C(CH_3)_3$), 0.12, 0.11, 0.04 and −0.17 (4s, 4×$SiCH_3$); δ_C ($CDCl_3$) 163.6, 158.5, 150.4, 145.1, 139.3, 136.2, 136.0, 130.2, 130.1, 128.2, 128.0, 126.8, 117.3, 113.3, 113.2, 108.9, 86.8 (d, J=4.6 Hz), 85.9, 85.4, 73.5 (d, J=16.9 Hz), 72.6 (d, J=2.2 Hz), 64.8, 64.4, 58.3 (d, J=21.8 Hz), 55.3, 43.2, 43.1, 26.1, 25.8, 24.8, 24.7, 24.6, 24.5, 20.4 (d, J=8.0 Hz), 18.5, 17.9, 12.7, −4.7, −4.8, −5.1, −5.2; MALDI-HRMS: m/z 1041.4926 ($[M+Na]^+$, $C_{53}H_{79}N_4O_{10}PSi_2Na^+$ calcd 1041.4965).

Synthesis and purification of modified oligonucleotides. The oligomers **ON5–ON10**, **ON15**, **ON17–ON19**, **ON21**,

ON23 and **ON24** (Table 1 and 2) were synthesized in 0.2 μmol scale on CPG solid support on an automated DNA-synthesizer using the phosphoramidite approach.[30] The stepwise coupling yield for the α-L-RNA phosphoramidites (T-monomer,[6] **6**, **12** and **25**) was above 90% (1*H*-tetrazole as activator, 20 min coupling time), for the phosphoramidite **34** approximately 88% (1*H*-tetrazole as activator, 60 min pre-activation by mixing **34** and activator, 120 min coupling time), and for phosphoramidite **43** approximately 95% (pyridinium chloride as activator, 10–16 min coupling time). After detritylation with 80% aq acetic acid, cleavage from the solid support and deacylations were effected using 40% aqueous methylamine (10 min, 55 °C). After cooling to −18 °C, the solid support was removed (centrifugation), washed [2×0.25 cm^3; EtOH–CH_3CN–H_2O (3/1/1, v/v/v)], and the combined liquid phase evaporated to dryness under reduced pressure. Desilylation of the oligomers was accomplished using a method described earlier[31] for 20 h (at 55 °C) and precipitation was then performed from *t*-BuOH. Standard conditions of the synthesizer were used for incorporation of DNA monomers whereas the incorporation of α-L-LNA monomers followed procedures described earlier.[2,3] The composition of the ONs was verified by MALDI-MS (negative ion mode) on a Micromass Tof Spec E mass spectrometer using a matrix of diammonium citrate and 2,6-dihydroxyacetophenone. Analysis by capillary gel electrophoresis verified the purity of the oligomers as being >80%. MALDI-MS of selected ONs *m/z* ($[M-H]^-$, found/calcd): **ON5**, 2800/2799; **ON6**, 2894/2891; **ON7**, 2800/2799; **ON8**, 2893/2891; **ON9**, 2900/2899; **ON10**, 2992/2987; **ON17**, 4238/4231; **ON18**, 4235/4231; **ON19**, 4237/4231; **ON24**, 4241/4242.

Thermal denaturation studies. Melting temperatures (T_m values, °C) were determined by measuring the absorbance at 260 nm against increasing temperature (1.0 °C/min) on equimolar mixtures (1.0 or 1.5 μM in each strand) of modified ONs and their complementary DNA/RNA strand in 10 mM phosphate buffers with different NaCl concentration (see captions to Tables 2 and 3) and were performed on a Perkin-Elmer UV–vis spectrometer fitted with a PTP-6 temperature programmer.

Molecular modelling. A DNA duplex of sequence 5′-d(GTGATATGC) and a DNA:RNA hybrid NMR solution structure[32] were used as template and further modified within the MacroModel V8.0 program suite.[33] The modified residue was first partially optimized (MMFF94s force field, 1000 cycles) and subsequently submitted to a 5 ns stochastic dynamics (300 K, 2 fs timestep, 1000 structures were sampled and minimized) using the SHAKE algorithm to keep X–H bond lengths fixed during the simulation. The non-bonded cut-off was 9 Å and a dielectric constant of 80 was applied. The residues neighbouring the modified residue were constrained whereas all other residues were frozen during stochastic dynamics.

RNase H assay. The 5′-r(AGGUCCAUAGAGAC) RNA target sequence was [^{32}P]-labelled at the 5-end with T4 kinase and the radioactive RNA was mixed with unlabelled RNA. 0.2 μM RNA (1 pmol/final sample) was incubated in the presence of a four-fold excess of complementary **ON16**, **ON17**, **ON18** or **ON19** in hybridization buffer (20 mM Tris–HCl, pH 7.5, 100 mM KCl) at 65 °C for 2 min followed by slow cooling to 37 °C. The RNase H digest was performed in 20 mM Tris–HCl, pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 1 mM with 0.01 U of *E. coli* RNase H (Amersham) enzyme at 37 °C. Aliquots of 10 μL samples were withdrawn and mixed with 5 μL formamide loading dye with 10 mM EDTA on ice at the time points 2, 10 and 60 min after RNase H addition. A basic hydrolysis of labelled RNA was performed by heating to 90 °C for 15 min in 100 mM Na_2CO_3 (pH 9.0, 2 mM EDTA) followed by cooling on ice and addition of formamide dye. All reaction products were analyzed by PAGE (20% polyacrylamide containing 8.3 M urea). The radioactive RNA bands were visualized by autoradiography of the dried gels.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.12.007. H contains copies of ^{13}C NMR spectra of compounds **1–3**, **5**, **7–11**, **14–20**, **22–24**, **27–33**, **36–42** and **43** (major isomer) and ^{31}P NMR spectra of compounds **6**, **12**, **25**, **34** (major isomer), **34** (minor isomer), **43** (major isomer) and **43** (minor isomer).

References and notes

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