XNA[‡] (*xylo* Nucleic Acid): A Summary and New Derivatives

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Fully modified homopyrimidine 2′-deoxy-x*ylo* nucleic acid (dXNA) form triple helixes with complementary DNA/RNA with thermal stabilities comparable to those of the corresponding DNA:DNA and DNA:RNA duplexes. However, a single or few insertions of dXNA monomers in a DNA strand significantly lower duplex stabilities. The dXNA monomers are known to adopt predominantly an *N*-type furanose conformation in solution. With a desire to increase the binding affinity, seven sugar-modified XNA monomers (**H**, **F**, **N**, **M**, **K**, **P** and **Q**) have been synthesised and their effect on hybridization towards DNA and RNA complements studied. The introduction of 2′-fluoro and 2′-hydroxy substituents was expected to induce conformational restriction towards C3′-*endo*-type furanose conformation of monomer **F** derived from 1-(2′-deoxy-2′-fluoro-β-D-xylofuranosyl)thymine and monomer **H** derived from 1-(β-D-xylofuranosyl)thymine. The presence of functionalites facing the minor groove as in 1-(2′-amino-2′-deoxy-2′-*N*,4′-*C*-methylene-β-D-xylofuranosyl)thymine (monomer **N**), 1-[4-*C*-(*N*-methylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (monomer **P**), 1-(4-*C*-piperazinylmethyl-β-D-xylofuranosyl)thymine (monomer **Q**), 1-(4-*C*-hydroxymethyl-β-D-xylofuranosyl)thymine (monomer **M**) and 9-(4-*C*-hydroxymethyl-β-D-xylofuranosyl)adenine (monomer **K**) was studied, with monomer **N** being locked in an *N*-type furanose conformation. Besides, an efficient synthesis of known x*ylo*-LNA phosphoramidite **19**, required for the incorporation of 1-(2′-*O*,4′-*C*-methylene-β-D-xylofuranosyl)thymine (monomer **L**) is described. For comparison, hydridization data of various XNAs reported in the literature are included in the discussion section. The thermal denaturation studies show that XNAs containing conformationally locked monomers (**N** and **L**) display improved binding affinity, and that partially modified DNA/XNA chimera, or fully modified XNA display preferential hybridization towards RNA complements.

Introduction

2′-Deoxy-*xylo*-configured oligonucleotides (2′-deoxy-*xylo* nucleic acid, dXNA)[1–6] form complexes with DNA/RNA complements that substantially differ from the unmodified counterparts in structure due to the inversion of the configuration at C3′ of the *xylo* monomers (3′*R* configuration) as compared to regular DNA/RNA monomers (3′*S* configuration). The dXNA monomers predominantly adopt an *N*-type furanose conformation as opposed to the *S*-type of their 2′-deoxy-*ribo* counterparts.[1] Notably, oligomers containing dXNA monomers possess a higher stability towards exonucleases.[1,2,5] A single replacement of the regular 2′-deoxynucleotides by their *xylo*-configured counterparts results generally in a significant decrease in the duplex stability.[2] However, with the RNA complement, the thermal stability of the duplex was comparable with the reference DNA:RNA duplex.[7,8] The increased binding affinity towards RNA has been attributed to the tendency of these nucleotides to adopt a C3′-*endo* conformation, giving thermally stable A-type duplexes. This hypothesis was confirmed by the thermal denaturation studies of *xylo*-LNA,[9,10] that contains monomer with sugar conformation locked in an *N*-type, leading to preferential hybridization towards RNA.

Whereas the introduction of a few dXNA monomers into a DNA strand had a very negative influence on the hybridization properties,[2,3,7,8,11] an almost fully modified pyrimidine dXNA displayed comparable, or even significantly increased, binding affinity towards DNA or RNA complements when compared to the reference duplex,[1,2,5–8] and a fully modified self-complementary dXNA formed a com-

[‡] We have defined an XNA as an oligonucleotide containing one or more nucleotide monomers with a xylofuranosyl configuration and internucleotide phospho diester linkages linked via the O3′ and O5′ atoms of the monomers.

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plex of similar stability to that formed by the corresponding DNA.[5] Recently, we[7,8] and Bertrand et al.[12] have shown that dXNA are able to form higher order complexes (i.e. triplexes) with single-stranded DNA and RNA targets. Gel retardation experiments,[7,12] UV melting experiments[7,8,12] and circular dichroism studies[11] have shown that in the triple helices formed by homopyrimidine dXNAs with complementary DNA or RNA strands, one dXNA strand is antiparallel (Watson–Crick pairing) and the other parallel (Hoogsteen pairing) with respect to the DNA/RNA target. Surprisingly, neither antiparallel nor parallel duplex formation between homopyrimidine dXNAs and their DNA/RNA targets was observed. The triplexes formed were stable at pH 7, but less stable than at pH 5.[12]

The main objective of the present work is to present an overview of known sugar-modified XNAs, and evaluate the opportunities to improve the binding affinity of XNA by either restricting their furanose conformation in an *N*-type or by covalently attaching functionalities to the XNA.[7] We undertook the synthesis of *xylo*-configured RNA-like monomer **H**, conformationally restricted 2′-deoxy-2′-fluoro XNA monomer **F** and conformationally locked 2′-amino-*xylo*-LNA monomer **N**. In addition we report a convenient synthesis of the precursor amidite **19** for the incorporation of *xylo*-LNA monomer **L**. We speculated that the introduction of an electronegative fluoro substituent at the 2′ position of dXNA monomer could increase the contribution from the *N*-type furanose conformation due to stereoelectronic effect, and that the presence of the secondary amine functionality in 2′-amino-*xylo*-LNA would be of potential interest as a conjugation site and as a protonation site.

Oligonucleotides containing 4′-*C*-hydroxymethyl nucleotide monomers hybridize with both complementary DNA and RNA with similar binding affinity as the corresponding unmodified DNA strand.[15,16] The additional *C*-alkyl branch faces the minor groove allowing attachment of various molecular entities,[16,17] e.g., intercalators, lipophilic groups, amines or a third strand. Incorporation of 4′-*C*-(hydroxymethyl)uridine into an oligonucleotide resulted in moderate to strong destabilization of the duplex with complementary DNA, whereas only a small decrease in the melting temperature was observed with complementary RNA as target.[18] This, combined with the preferential hybridization of dXNA towards RNA complements relative to DNA complements, inspired the synthesis of 4′-*C*-hydroxymethyl *xylo*-configured RNA-like monomers **M** and **K** (Figure 1). Furthermore, oligonucleotides containing 4′-*C*-substituted nucleotides have shown increased resistance towards enzymatic degradation,[15,18,19] most pronounced for oligonucleotides containing modified nucleotides with an amino group.[20,21] Besides, as the 4′α position of the nucleotides is close to the internucleotide phospho diester linkages, the attachment of alkylamines anticipated to be protonated under physiological conditions is attractive as a strategy for increasing the binding affinity towards the negatively charged target strands. Recently, based on similar considerations, we have successfully introduced basic piperazinyl moieties, pointing either towards the minor or major groove of duplexes.[22] Thus, in an attempt to increase the binding affinity of XNA, we decided to synthesize and evaluate oligonucleotides containing 4′-*C*-piperazinylmethyl XNA monomers **P** and **Q**.

Results and Discussion

The known dXNA thymine phosphoramidite **1** was prepared from thymidine following the reported procedure[1,23] and used for the incorporation of monomer **X** into oligonucleotides.

Synthesis of 2′-Fluoro-dXNA Monomer F:[7] Conversion of known methyl 2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-β-D-

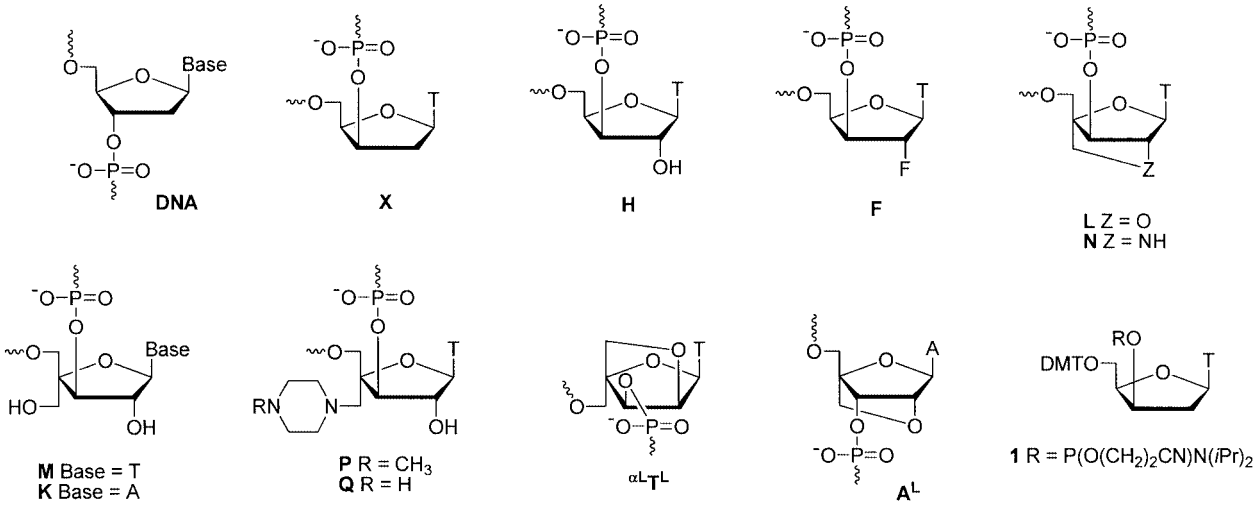
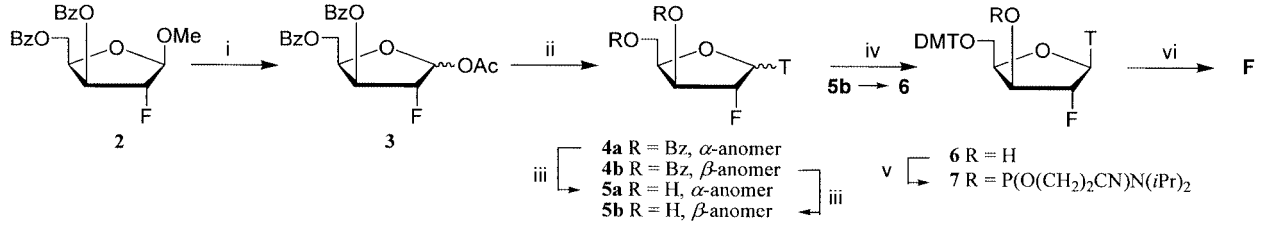


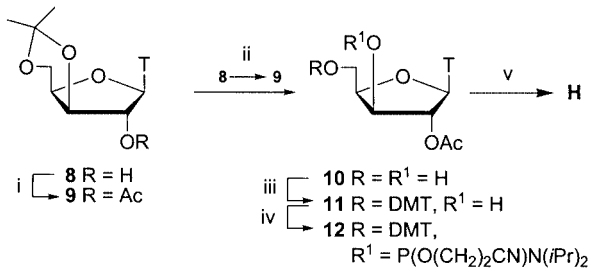
Figure 1. Structures of nucleotide monomers studied: DNA, dXNA thymine monomer (**X**), XNA thymine monomer (**H**), 2′-deoxy-2′-fluoro XNA thymine monomer (**F**), *xylo*-LNA thymine monomer (**L**), 2′-amino-*xylo*-LNA thymine monomer (**N**), 4′-*C*-hydroxymethyl XNA monomers (**M** and **K**), 4′-*C*-piperazinylmethyl XNA monomers (**P** and **Q**), α-L-LNA thymine monomer ($^{\alpha L}T^{L}$)[9,13] and LNA adenine monomer (A^{L}).[14] The short notations shown are used in Tables 1–3. T = thymin-1-yl, A = adenin-9-yl.



Scheme 1. Reagents and conditions (yields): (i) AcOH/Ac_2O/concd. H_2SO_4 (3.8:1:0.3, v/v/v), room temp., 75 min (97%); (ii) thymine, BSA, TMS-triflate, 1,2-dichloroethane, reflux, 3 h (**4a**: 24%, **4b**: 49%); (iii) saturated methanolic ammonia, room temp., 12 h (**5a**: 75%, **5b**: 83%); (iv) DMTCl, pyridine, room temp., 14 h (90%); (v) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, room temp., 30 min. (81%); (vi) DNA synthesizer.

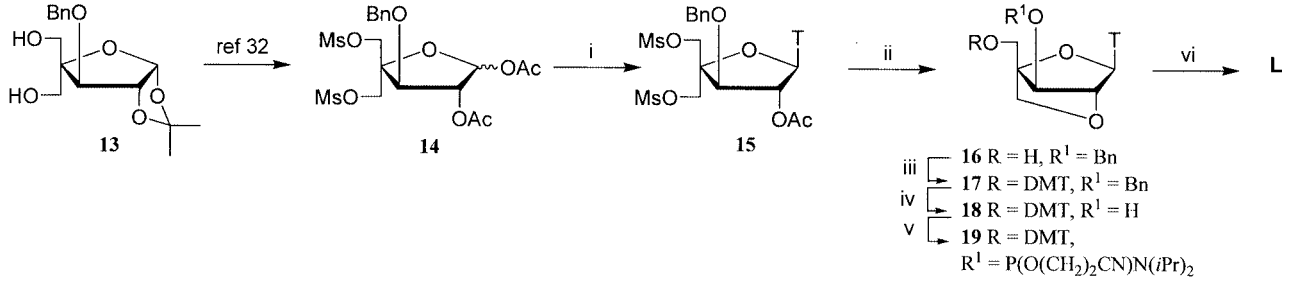
xylofuranoside (**2**)[24] into a suitable phosphoramidite building block for synthesis of XNAs with monomeric unit **F** was performed as depicted in Scheme 1. Acetolysis of **2** afforded the anomeric mixture **3**, which was coupled with bis-*O*-(trimethylsilyl)thymine in the presence of TMS-triflate to give the isomers **4** (α-anomer **4a** and β-anomer **4b**, in a ratio **4a**/**4b** = 1:2 after isolation as individual compounds by column chromatography) in a total yield of 73%. Deprotection of nucleosides **4a** and **4b** with saturated ammonia in methanol afforded α-anomer **5a** and β-anomer **5b** in 75% and 83% yields, respectively. The anomeric configuration of **5a** and **5b** was assigned by ^1H NMR spectroscopy. For the β-anomer **5b**, the signal of H1′ (δ, 6.02) appeared as a doublet with a large coupling constant ($^3J_{H1',F}$ = 21.4 Hz). The lack of a significant coupling between protons H1′ and H2′ is characteristic of a *trans*-1′,2′-configuration in furanose derivatives,[25] thus confirming the β-configuration. On the other hand, in the α-anomer **5a**, the signal of H1′ (δ, 6.24) appeared as a double doublet with a significant coupling constant between H1′ and H2′ ($^3J_{H1',H2'}$ = 3.3 Hz). Moreover in the spectrum of **5a**, the signal for H4′ is shifted ≈0.4 ppm downfield when compared to the corresponding signal in the spectrum of **5b** which further supports the anomeric assignments.[26] Selective protection of the 5′-hydroxy group of diol **5b** by the treatment of DMTCl in pyridine gave nucleoside **6** in 90% yield. The H1′ signal of nucleoside **6** appears as a doublet ($^3J_{H1',F}$ = 21.1 Hz), and the absence of $^3J_{H1',H2'}$ coupling confirms the configurational assignments and indicates an *N*-type furanose conformation[27,28] for **6** (and therefore also for monomer **F**). Phosphitylation of **6** by the standard protocol afforded phosphoroamidite **7** (81% yield) that was used to incorporate monomer **F** into oligonucleotides.

Synthesis of XNA Monomer H: For the synthesis of XNAs with monomeric unit **H**, 1,2-*O*-isopropylidene-α-D-xylofuranose was selected as the starting material, and it was converted in five steps in 42% overall yield to 1-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)thymine (**8**) following the published procedure.[29] The 2′-hydroxy group was acetylated in 97% yield (to give **9**) and the 3′,5′-isopropylidene group was cleaved by refluxing with 80% aq. AcOH to afford nucleoside **10**. Selective dimethoxytritylation at the primary hydroxy group of **10** by reaction with DMTCl in the presence of pyridine and subsequent phosphitylation furnished the desired phosphoramidite **12** (78% yield, two steps) suitable for incorporation of monomer **H** into oligonucleotides (Scheme 2).



Scheme 2. Reagents and conditions (yields): (i) Ac_2O, pyridine, room temp., 12 h (97%); (ii) 80% aq. AcOH, 65 °C to room temp., 14 h (84%); (iii) DMTCl, pyridine, room temp., 12 h (90%); (iv) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, room temp., 30 min. (87%); (v) DNA synthesizer.

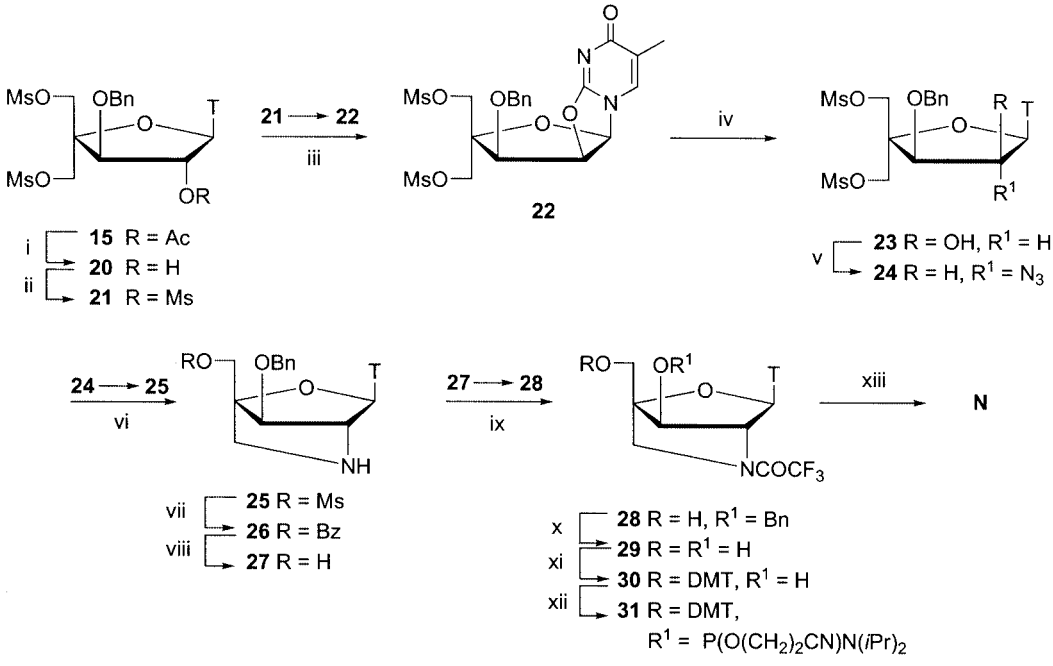
New Strategy for Synthesis of *xylo*-LNA Monomer L: We have earlier reported the synthesis of phosphoramidite **19**[9,30] that was used for incorporation of *xylo*-LNA monomer **L** into oligonucleotides. Although significant duplex destabilization by the introduction of one or few *xylo*-LNA monomers **L** into a DNA strand was observed, strong binding affinity was obtained for an almost fully modified *xylo*-LNA towards DNA or RNA complements[10] (T_m +3.1 and +4.3 °C per modification, respectively), which has not been reported for other XNA derivatives. Inconveniently phosphoramidite **19** was synthesized from 3-*O*-benzyl-4-*C*-hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose (**13**)[31] in an overall yield of only 3.3%.[30] Therefore, we have developed a new strategy for the synthesis of this interesting phosphoramidite that significantly improves the overall yield to 14.3% (Scheme 3). Furanose **14** was obtained from **13** in 50% yield following the known literature procedure.[32] Coupling of **14** with thymine in the presence of BSA and TMS-triflate in acetonitrile afforded nucleoside **15** in 72% yield. Subsequent refluxing of **15** with a large excess of NaOH in EtOH afforded nucleoside **16**, as a result of a reaction cascade involving first 2′-deacetylation, then intramolecular ring closure and exchange of the mesyloxy group at C5′ with a hydroxy group. Nucleoside **16** was converted by the standard protocol into its 5′-dimethoxytrityl derivative **17**. It has been previously reported that hydrogenolysis of **17** in the presence of Pd/C in MeOH resulted in concomitant detritylation.[30] However, when the reaction was car-



Scheme 3. Reagents and conditions (yields): (i) thymine, BSA, TMS triflate, acetonitrile, reflux, 3 h (72%); (ii) aq. NaOH (2 M), 96% EtOH, reflux, 60 h (76%); (iii) DMTCl, pyridine, room temp., 12 h (95%); (iv) H_2, $Pd(OH)_2$, EtOAc/EtOH, 70 °C, 28 h (67%); (v) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, room temp., 30 min. (82%); (vi) DNA synthesizer.

ried out with $Pd(OH)_2$/C in a mixture of EtOAc/EtOH, detritylation was avoided and nucleoside **18** was obtained in 67% yield. Subsequent conversion of **18** into the phosphoroamidite **19** completed the optimized synthesis of the building block needed for the incorporation of *xylo*-LNA monomer **L** into oligonucleotides.

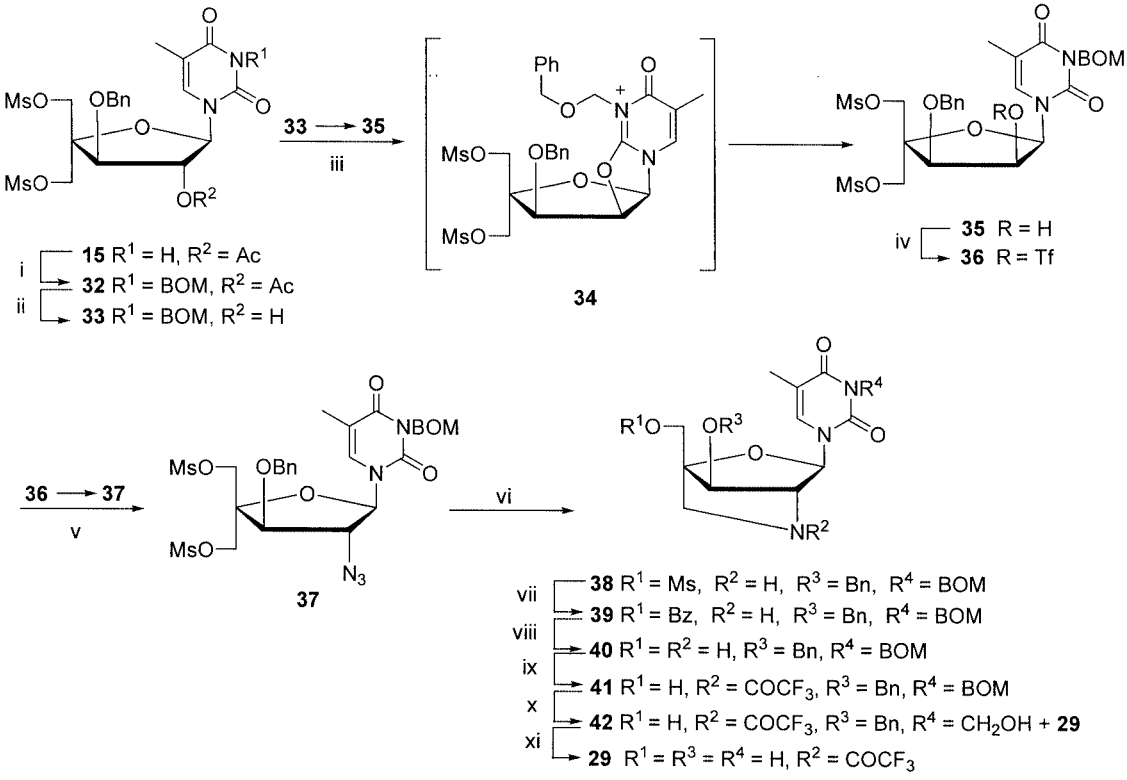
Synthesis of 2′-Amino-*xylo*-LNA Monomer N:[7] The synthesis of 2′-amino-*xylo*-LNA phosphoramidite building block **31** was carried out in twelve steps in an overall yield of 13%, starting from nucleoside **15** (Scheme 4). Deprotection of the O2′ acetyl group was carried out using saturated methanolic ammonia affording nucleoside **20** in 93% yield. In order to obtain the (2′*R*)-2′-azido nucleoside **24**, a double inversion strategy was applied. The first inversion at the 2′ position was carried out using a two-step procedure, i.e., mesylation of the secondary hydroxy group followed by displacement of the newly formed mesylate in **21** taking advantage of the nucleophilic nature of the O2 position of the nucleobase, affording the 2,2′-anhydro nucleoside **22**. Opening of the 2,2′-anhydro moiety with retention of configuration at C2′ was done by refluxing **22** in a mixture of aqueous sulfuric acid (0.4 M) and acetone (1:1, v/v), which resulted in *erythro*-configured nucleoside **23** in 98% yield. The second inversion was achieved by activation of the 2′-OH in **23** by reaction with 1.1 equivalents of trifluoromethanesulfonic anhydride and subsequent nucleophilic displacement by azide furnishing *threo*-configured nucleoside **24**. Reduction of the azido group in nucleoside **24** under modified Staudinger conditions using trimethylphosphane in a mixture of THF and aqueous NaOH and simultaneous in situ intramolecular cyclization afforded the bicyclic nucleoside **25** in 53% yield from **23**. Nucleophilic displacement of the remaining mesyloxy group in **25** with a benzoate group, followed by ester cleavage with saturated meth-



Scheme 4. Reagents and conditions (and yields): (i) saturated NH_3 in MeOH, 0 °C to room temp., 45 min (93%); (ii) MsCl, pyridine, room temp., 4 h (86%); (iii) DBU, CH_3CN, room temp., 1.5 h (89%); (iv) 0.4 M aq. H_2SO_4, acetone, reflux, 14 h (98%); (v) a) Tf_2O, pyridine, DMAP, CH_2Cl_2, 0 °C, 7 h; b) NaN_3, DMF, room temp., 18 h; (vi) PMe_3, aq. NaOH (2 M), THF, room temp., 72 h (53% from **23**); (vii) NaOBz, 15-crown-5, DMF, 120 °C, 30 h; (viii) saturated NH_3 in MeOH, room temp., 18 h (48%, 2 steps); (ix) CF_3CO_2Et, DMAP, MeOH, room temp., 16 h (93%); (x) 10% Pd/C, H_2, MeOH, room temp., 2 h (96%); (xi) DMTCl, pyridine, room temp., 18 h (94%); (xii) $NC(CH_2)_2OP(Cl)N(iPr)_2$, DIPEA, CH_2Cl_2, room temp., 8 h (90%); (xiii) DNA synthesizer.

anolic ammonia afforded nucleoside **27**. Protection of the secondary amino group using ethyl trifluoroacetate, followed by debenzylation, using catalytic hydrogenation with palladium as the promoter (10% Pd/C), afforded diol **29**, as a (1:2) mixture of rotamers. To prepare for automated synthesis of XNAs containing 2′-amino-*xylo*-LNA monomer **N**, the primary hydroxy group of nucleoside **29** was selectively dimethoxytritylated followed by phosphitylation under standard conditions to furnish the desired phosphoramidite building block **31**. The assigned configuration of nucleoside **29** was confirmed by NOE difference experiments. Thus selective irradiation of the signal for H5′ protons gave a 3% enhancement in the signals of H6 and 3′-OH. In addition mutual NOE effects were observed between H1′ and H5′′ (1%/1%), H3′ and H5′′ (2%/1%) and between H6 and 3′-OH (2%/1%), which besides verifying the configuration, also suggest that the bicyclic nucleoside adopts the expected *N*-type (C3′-*endo*) furanose conformation.

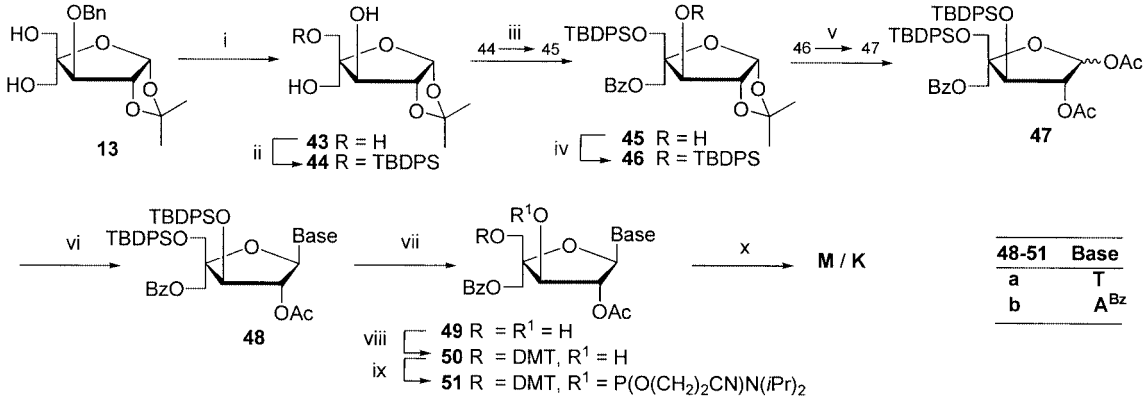
Alternative Synthesis of 2′-Amino-*xylo*-LNA Diol 29: The diol **29** was synthesized from nucleoside **15** in an overall yield of 16% (Scheme 4). This yield was improved to 21% by following an alternative synthetic route (Scheme 5), the key step being a one-pot inversion reaction (**35** from **33**) serendipitously discovered in our laboratories. The synthesis of the 2′-amino-*xylo*-LNA phosphoramidite building block **31** from starting material **15** was hereby achieved in an overall yield of 18%. Nucleobase protection of compound **15** at the N3 position was carried out with benzyloxymethyl chloride (BOMCl) in the presence of diisopropylethylamine to give nucleoside **32** in 86% yield after purification by dry column vacuum chromatography (DCVC).[33] Nucleoside **32** was stirred in saturated methanolic ammonia at 0 °C and then warmed to room temperature over a period of 15–20 min affording the deacetylated nucleoside **33** in 97% yield. Reaction of alcohol **33** with trifluoromethanesulfonic anhydride in the presence of pyridine (10 equivalents) and DMAP (4 equivalents) in CH_2Cl_2 at 0 °C, followed by the addition of saturated aqueous $NaHCO_3$, surprisingly resulted in the C2′ inverted alcohol **35** in 72% yield. The inversion is most likely a consequence of in situ 2,2′-anhydro formation (intermediate **34**), followed by hydrolysis with retention of configuration. For the synthesis of the desired *threo*-configured 2′-amino *xylo*-LNA intermediate another epimerization at C2′ was required. Accordingly, activation of the 2′-OH of **35** by reaction with trifluoromethanesulfonic anhydride and subsequent substitution with an azide afforded nucleoside **37** in 58% yield from **35**. Reduction of the azide group of nucleoside **37** was carried out by Staudinger reaction, using triphenylphosphane in pyridine for 20 h at room temperature followed by the addition of concentrated aqueous ammonia to hydrolyze the intermediate



Scheme 5. Reagents and conditions (and yields): (i) BOMCl, DIPEA, CH_2Cl_2, 0 °C to room temp., 44 h (86%); (ii) saturated NH_3 in MeOH, 0 °C to room temp., 15 min (94%); (iii) a) Tf_2O, pyridine, DMAP, CH_2Cl_2, 0 °C, 4 h; b) saturated aqueous $NaHCO_3$ (72%); (iv) Tf_2O, pyridine, DMAP, CH_2Cl_2, 0 °C, 4 h (70%); (v) NaN_3, DMF, room temp., 18 h (83%); (vi) a) PPh_3, pyridine, room temp., 20 h; b) concd. aqueous NH_3, room temp., 24 h (99%); (vii) NaOBz, 15-crown-5, DMF, 120 °C, 24 h (92%); (viii) saturated NH_3 in MeOH, room temp., 20 h (93%); (ix) CF_3CO_2Et, DMAP, MeOH, room temp., 4 h (78%); (x) a) BCl_3, CH_2Cl_2, –78 °C to room temp., 2 h; b) H_2O, room temp., 24 h (91%).

imino-phosphorane, which also resulted in intramolecular cyclization, affording the bicyclic nucleoside **38** in 99% yield. Displacement of the remaining mesylate in **38** by excess sodium benzoate in DMF at 120 °C, followed by cleavage of the benzoate ester in **39** with saturated methanolic ammonia, provided alcohol **40** in 86% yield (from **38**). The configuration and conformation of nucleoside **39** was unambiguously established by NOE difference experiment. Irradiation of the signal for H1′ proton resulted in enhancement in the signals of H5′′ (1%) and 2′-NH (4%). Besides, mutual NOE enhancement was observed between H3′ and H5′′ (2%/5%). Transesterification of **40** proceeded smoothly with excess (7 equivalents) of ethyl trifluoroacetate in the presence of 2.5 equivalents of DMAP in MeOH at room temperature, affording trifluoroacetyl-protected nucleoside **41** as a mixture of two rotamers in 78% yield. The simultaneous deprotection of the benzyl group at C3′ and the benzyloxymethyl group at N3 by catalytic hydrogenation failed. However, Lewis acid-catalyzed debenzylation using boron trichloride in CH_2Cl_2 resulted in a mixture of the desired nucleoside **29** and nucleoside **42** (tentatively assigned as shown in Scheme 5 but neither isolated nor characterized) in the ratio 1.0:1.5 as judged from NMR spectra of the crude mixture. The removal of the hydroxymethyl group at the N3 position of the nucleobase was achieved by stirring the crude mixture (**29** + **42**) in H_2O at room temperature for 24 h which resulted in complete conversion of **42** into **29**.

Synthesis of 4-*C*-Hydroxymethyl-XNA Monomers M and K: Synthesis of 4-*C*-hydroxymethyl-XNA monomers **M** and **K** was achieved by a convergent strategy in which the suitably protected xylofuranose **47** was condensed with the nucleobases under Vogbruggen conditions (Scheme 6). Furanose **13**[31] was selected as the starting material and efficient regioselective modification of the two primary hydroxy groups was critical for the synthesis. Benzylation of **13** using 1 equivalent of BnBr under various conditions gave a ca. 1:1 inseparable mixture of 3,5- and 3,5′-dibenzylated furanoses. Silylation with the bulky TBDPS group improved neither the regioselectivity nor the separation of the isomers by column chromatography. Similar reactivity of the two primary hydroxyls has been attributed to their presence in similar environment.[34] The hydroxy group at C5′ on the concave face of the bicyclo[3.3.0]octane system is expected to be less reactive than the 5-OH on the convex face, while the bulky benzyloxy group at C3 limits the reactivity of the 5-OH for steric reasons. We therefore envisioned that removal of the benzyl protection at C3 would enhance the reactivity of 5-OH group. This hypothesis was proven to be true when the triol **43**[35] was reacted with 1.1 equivalent of TBDPSCl resulting in the 5-*O*-silylated furanose **44** being obtained in almost quantitative yield. Surprisingly the formation of the regioisomeric O5′-silylated product was not observed while traces of disilylated product and unreacted triol **43** were seen on analytical TLC. The unreacted triol **43** was removed by washing the reaction mixture with saturated aq. $NaHCO_3$, whereas the disilylated product was removed by quick fractionation through a silica gel column. The configuration of **44** was determined by NOE difference spectroscopy, and was later confirmed by a single-crystal X-ray diffraction study on furanose **54a**[36] (Figure 2). Selective irradiation of the signal for H5 of compound **44** induced a 4% enhancement of the signal for 3-OH and vice-versa (1%), which suggested that these protons are positioned at the same side of the furanose ring. Moreover, significant NOE contacts were observed between H3, H5′ and 5′-OH (3.2%/5.0% between H3 and H5′a, 3.0%/3.4% between H3 and H5′b, 2.1/2.2% between H3 and 5′-OH) confirming *cis* positioning of these protons on the furanose ring. The next step was the protection of the remaining hydroxy groups under mild basic conditions (to avoid silyl migration). Selective O5′-benzoylation of diol **44** followed by O3-silylation afforded furanose **46** in 80% yield. In situ acetolysis and acetylation with Ac_2O and AcOH in the presence of catalytic amounts of concd. H_2SO_4 afforded furanose **47** which served as a common gly-



Scheme 6. Reagents and conditions (yields): (i) HCO_2NH_4, $Pd(OH)_2/C$, MeOH, reflux, 16 h (89%); (ii) TBDPSCl, Et_3N, CH_2Cl_2, room temp., 36 h (93%); (iii) BzCl, pyridine, CH_2Cl_2, 0 °C to room temp., 12 h (86%); (iv) TBDPSCl, imidazole, DMAP, anhydrous DMF, room temp., 12 h (93%); (v) Ac_2O, AcOH, concd. H_2SO_4, room temp., 4 h (87%); (vi) thymine, BSA, TMS-triflate, acetonitrile, reflux, 12 h (**48a**: 91%) or 6-*N*-benzoyladenine, $SnCl_4$, acetonitrile, reflux, 36 h (**48b**: 66%); (vii) TBAF, THF, room temp. (**49a**: 93%, **49b**: 52%); (viii) DMTCl, pyridine, room temp., 12 h (**50a**: 81%, **50b**: 88%); (ix) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, room temp., 12 h (**51a**: 83%, **51b**: 86%); (x) DNA synthesizer.

cosyl donor. Condensation of **47** with silylated thymine in the presence of TMS-triflate afforded the desired *xylo*-configured nucleoside **48a**. Desilylation of **48a**, followed by O5′-dimethoxytritylation to give **50a** and then O3′-phosphitylation, using standard procedures, furnished the phosphoramidite building block **51a** (Scheme 6) suitable for the incorporation of monomer **M** into oligonucleotides. The corresponding adenosine derivative was prepared following the same synthetic route, except that the condensation of the silylated 6-*N*-benzoyladenine with **47** required longer reaction time using $SnCl_4$ as Lewis acid furnishing nucleoside **48b** in moderate yield. In order to obtain oligonucleotides containing monomer **K**, desilylation of **48b**, followed by O5′-dimethoxytritylation and then O3′-phosphitylation afforded the desired phosphoramidite **51b** (Scheme 6).

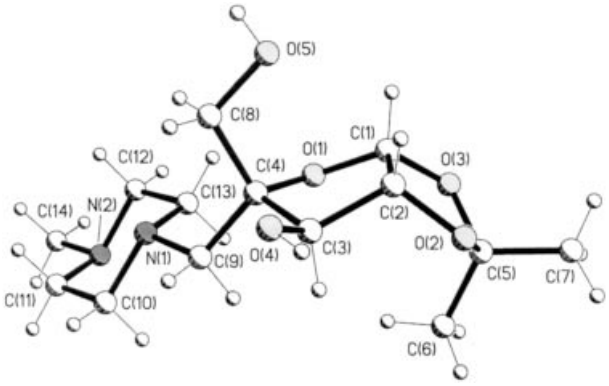
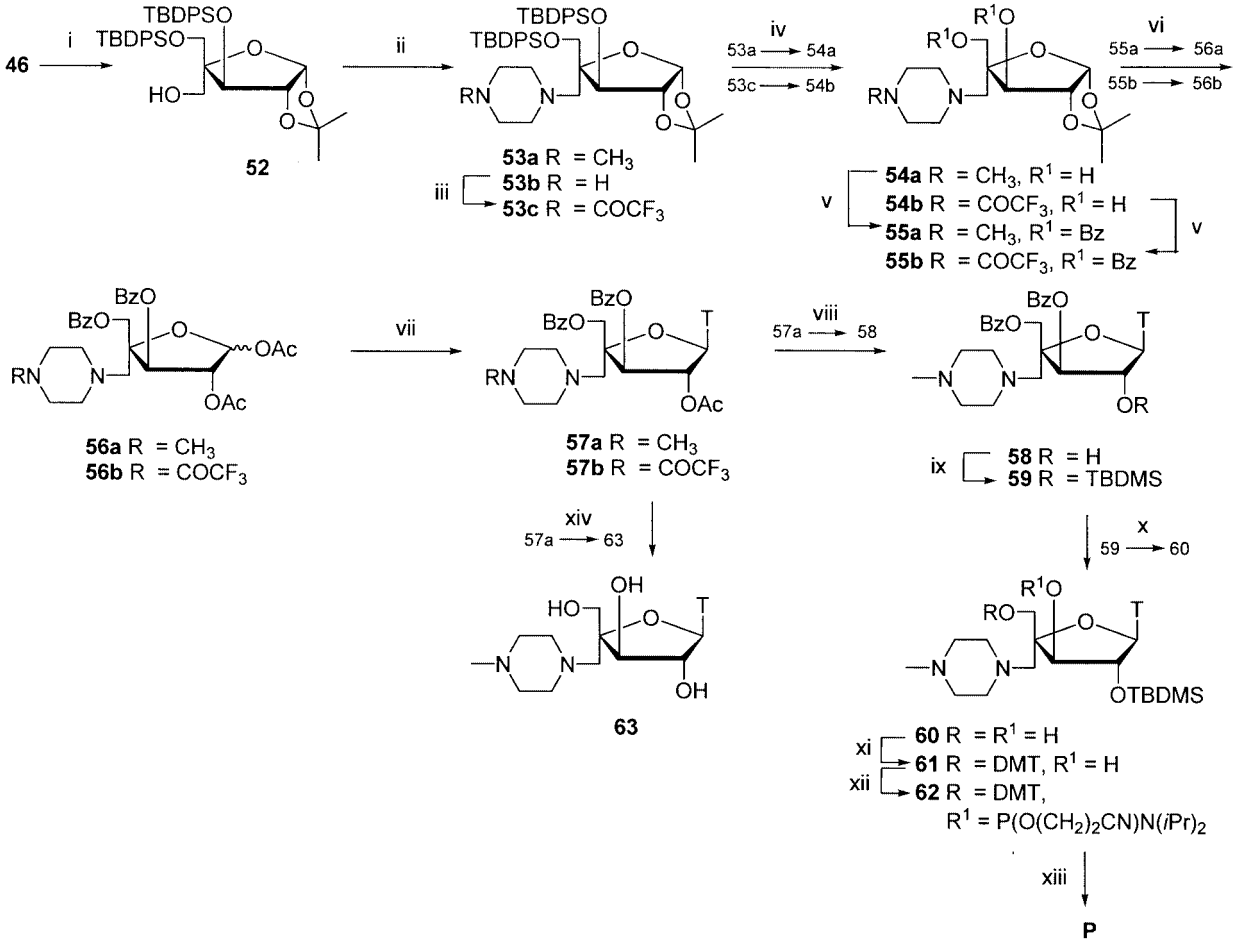
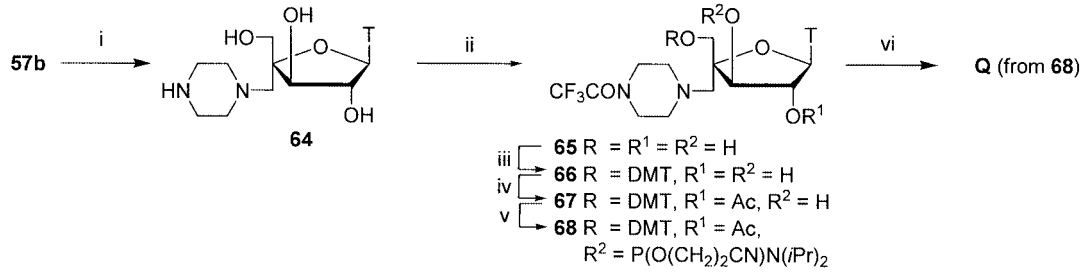


Figure 2. Molecular structure (ORTEP plot) of **54a**.[36] Only the numbering of the furanose ring follows the numbering otherwise used in this text.

Synthesis of 4-*C*-Piperazinylmethyl-XNA Monomers P and Q: For the synthesis of 4′-*C*-piperazinylmethyl-XNA monomers **P** and **Q**, furanose **52** was selected as the common precursor obtained by debenzoylation of **46** (Scheme 7). Activation of the 5′-hydroxy group by reaction



Scheme 7. Reagents and conditions (yields): (i) Saturated methanolic ammonia, room temp., 24 h (90%); (ii) a) Tf_2O, pyridine, CH_2Cl_2, –30 °C to room temp., 12 h; b) *N*-methylpiperazine, THF, room temp., 12 h (**53a**: 93%) and (ii) a) Tf_2O, pyridine, CH_2Cl_2, –30 °C to room temp., 12 h; b) piperazine, THF, room temp., 12 h (**53b**: 91%); (iii) $(CF_3CO)_2O$, Et_3N, CH_2Cl_2, room temp., 4 h (87%); (iv) TBAF, THF, room temp., 12 h (**54a**: 88%); (v) BzCl, pyridine, CH_2Cl_2, room temp., 12 h (**55a**: 87%, **55b**: 84% from **53c**); (vi) a) 80% aq. TFA, room temp., 3 h; b) Ac_2O, pyridine, room temp., 12 h (**56a**: 93%, **56b**: 91%); (vii) thymine, BSA, TMS-triflate, acetonitrile, reflux, 3 h (**57a**: 76%, **57b**: 83%); (viii) 10% saturated methanolic ammonia, room temp., 1 h [**58**: 57%, **57a**: 30% (recovered)]; (ix) TBDMSCl, imidazole, DMAP, anhydrous DMF, 36 °C, 12 h (86%); (x) saturated methanolic ammonia, room temp., 24 h (83%); (xi) DMTCl, pyridine, room temp., 12 h (88%); (xii) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, room temp., 6 h (91%); (xiii) DNA synthesizer; (xiv) saturated methanolic ammonia, room temp., 36 h (65%).



Scheme 8. Reagents and conditions (yields): (i) Saturated methanolic ammonia, room temp., 36 h (71%); (ii) ethyl trifluoroacetate, DMAP, MeOH, room temp., 12 h (84%); (iii) DMTCl, pyridine, room temp., 12 h (89%); (iv) Ac_2O, DMAP, acetonitrile, room temp., 3 h (72%); (v) $NC(CH_2)_2OP(Cl)N(iPr)_2$, $EtN(iPr)_2$, CH_2Cl_2, room temp., 6 h (91%); (vi) DNA synthesizer.

with triflic anhydride followed by nucleophilic displacement with *N*-methylpiperazine or piperazine furnished **53a** and **53b**, respectively. The free amino group of **53b** was protected as its trifluoroacetamide derivative **53c** in 87% yield by reaction with trifluoroacetic anhydride. Cleavage of the 1,2-*O*-isopropylidene moieties in **53a** and **53c** could not be achieved under various conditions, e.g., Ac_2O/AcOH/concd. H_2SO_4, 80% aq. TFA or refluxing with 80% aq. AcOH (only desilylation was observed). However, after replacing the silyl groups with benzoyl groups via diols **54a/54b**, the isopropylidene moieties could be cleaved easily by treatment with 80% aq. TFA for 1 h at room temperature which on subsequent peracetylation afforded the anomeric mixtures **56a/56b**, respectively, suitable for condensation with nucleobases. Accordingly, the mixture of the anomeric furanoses **56a/56b** was stereoselectively coupled with silylated thymine under modified Vorbruggen conditions to afford nucleosides **57a** and **57b**, respectively. Selective O2′-deacetylation of nucleoside **57a** was performed with 10% saturated methanolic ammonia at room temperature affording **58** which upon silylation furnished nucleoside **59** in 49% overall yield from **57a**. Zemplen *O*-debenzoylation of **59** yielded the diol **60** in 83% yield, which was prepared for incorporation into oligonucleotides by standard dimethoxytritylation of the 5′-hydoxy group, followed by phosphitylation of the 3′-hydroxy group to give the phosphoramidite building block **62** suitable for the incorporation of monomer **P** into oligonucleotides. The corresponding fully deprotected nucleoside **63** was obtained in 65% yield by stirring **57a** in saturated methanolic ammonia for 36 h at room temperature. For structural confirmation, an X-ray structure of furanose **54a** was obtained[36] (Figure 2).

Efforts towards selective O2′-deacetylation of **57b** proved futile as the analysis of the reaction mixture by analytical TLC showed the formation of several products. Therefore **57b** was completely deacylated, and to avoid N-branching during oligomerization the resulting nucleoside **64** was treated with ethyl trifluoroacetate to give a 84% yield of trifluoroacetamide derivative **65**. Subsequent O5′-dimethoxytritylation followed by selective acetylation of the unhindered 2′-hydroxy group (to give nucleoside **67**) and then O3′-phosphitylation afforded the desired phosphoramidite **68** suitable for the incorporation of monomer **Q** into oligonucleotides (Scheme 8).

Synthesis of XNAs Containing Monomers X, H, M, K, P or Q: All XNAs **ON8**, **ON21**–**ON34**, **ON36**, **ON37**, **ON39** and **ON40** (Tables 1–3) were prepared in 0.2 μmol scale using the phosphoramidite approach (see the experimental section for details). The composition of XNAs was verified by MALDI-MS analysis and their purity (>80%) by capillary gel electrophoresis.

Thermal Denaturation Studies: In agreement with literature reports,[1–8,12] a single replacement of a DNA thymine monomer by its 2′-deoxy-XNA (dXNA) counterpart **X** destabilized the duplex by 6–10 °C when hybridized to complementary DNA (Table 1, **ON2** relative to **ON1**, **ON6** relative to **ON5**). Preferential binding towards the RNA complement was observed (destabilization of the duplex by only 1–4 °C), which can be attributed, in part, to furanose puckering of the dXNA in an *N*-type conformation.[1] Incorporation of a few isolated **X** monomers into a DNA strand significantly reduced the affinity towards DNA and RNA targets (**ON3** relative to **ON1**), and no cooperative transition above 5 °C could be detected. In contrast, efficient recognition of both DNA and RNA targets was achieved by an almost fully modified dXNA (**ON7**), still with preferential hybridization to the RNA target. Preference of dXNA for the RNA complement was confirmed when **ON7** was hybridized with the RNA-mimicking LNA target (T_m = 46 °C), the corresponding DNA-LNA duplex displayed a T_m of only 38 °C. Efficient binding of the fully modified pyrimidine XNAs with DNA or RNA complements has been shown[7,12] to be due to triplex formation in which the first XNA forms Watson–Crick base pairing with the target while the second XNA is engaged in Hoogsteen base pairing.

For further optimization of the binding properties of fully modified dXNA we synthesized the *xylo*-clamp **ON8** (Table 1) in analogy to similar DNA-clamp oligomers.[37] This molecule, designed for combined Watson–Crick and Hoogsteen recognition, hybridizes towards both DNA and RNA targets more efficiently than the 14-mer dXNA **ON7**. However, the *xylo*-clamp **ON8**, in contrast to dXNA **ON7**, does not display preferred recognition of RNA over DNA. The same tendency of increase of thermal stability is ob-

Table 1. Thermal[a] denaturation experiments of duplexes containing XNA monomers **X** and **F**.

		DNA target T_m (ΔT_m) [°C]	RNA target T_m (ΔT_m) [°C]	LNA target [5′-($\mathbf{A^L}$A)$_7$] T_m [°C]
ON1	5′-d(GTGATATGC)	30 (ref.)	26 (ref.)	
ON2	5′-d(GTGA**X**ATGC)	24 (–6)[7]	25 (–1)[7]	
ON3	5′-d(G**X**GA**X**A**X**GC)	n.t.[7]	n.t.[7]	
ON4	5′-d(GTGA**F**ATGC)	25 (–5)[7]	27 (+1)[7]	
ON5	5′-T_{14}	33 (ref.)	29 (ref.)	38
ON6	5′-T_7**X**T_6	23 (–10)[7,8]	25 (–4)[7,8]	
ON7	5′-$\mathbf{X}_{13}$T	33 (+0)[7,8]	38 (+9)[7,8]	46
ON8	5′-$\mathbf{X}_{14}$ACACA$\mathbf{X}_{14}$C	43 (+10)	42 (+13)	53
ON9	5′-T_7**F**T_6	23 (–10)[7,8]	25 (–4)[7,8]	
ON10	5′-$\mathbf{F}_{13}$T	36 (+3)[7,8]	36 (+7)[7,8]	
ON11	5′-$\mathbf{X}_2\mathbf{F}\mathbf{X}_3\mathbf{F}\mathbf{X}_3$ $\mathbf{F}\mathbf{X}_2$T	34 (+1)[7]	38 (+9)[7]	

[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 medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 μM concentrations of the two complementary strands (assuming identical extinction coefficients for all modified and unmodified oligonucleotides); A = adenin-9-yl monomer, C = cytosin-1-yl monomer, G = guanin-9-yl monomer, T = thymin-1-yl monomer; see Figure 1 for the structures of 2′-deoxy-XNA thymine monomer **X**, 2′-deoxy-2′-fluoro-XNA thymine monomer **F** and adenin-9-yl LNA monomer $\mathbf{A^L}$; "n.t.": No cooperative melting transition detected.

served for the complex formed between *xylo*-clamp **ON8** and its LNA complement. We anticipate that the complex formed with *xylo*-clamp **ON8** involves a bimolecular triplex, and that the increased thermal stability relative to **ON7** is due to more favorable entropic changes during triplex formation.

Next the thermal stability of the complexes involving 2′-fluoro-XNA monomer **F** was determined (Table 1). We envisioned that preorganization of the furanose conformation in an *N*-type would enable entropically favored duplex formation. However, in direct comparison with 2′-deoxy-XNA **X**, no favorable change in the duplex stability was seen for monomer **F**. A single insertion of monomer **F** leads to decreased affinity towards unmodified DNA (ΔT_m –5 to –10 °C, **ON4** relative to **ON1**, **ON9** relative to **ON5**), while the effect is less detrimental towards RNA (ΔT_m +1 to –4 °C).[7,8] The (almost) fully modified 2′-fluoro-XNA (**ON10**) display T_m values in the same range as that of the reference **ON1** and the corresponding dXNA (**ON5**).[7,8] Moreover, it is possible to combine monomer **F** with dXNA monomers and still obtain satisfactory hybridization, as shown for the chimeric XNA **ON11**.[7,8]

Detrimental effect on the thermal stability of duplexes was also seen by the introduction of a single *xylo*-LNA monomer **L** (Table 2),[9] even though the furanose conformation is locked in an *N*-type. Upon four consecutive incorporations of monomer (**L**) in the middle of a 14-mer (**ON17**), no transition above 5 °C could be detected.[10] Apparently, the unnatural *xylo*-configuration and phospho diester linkages are nonideal in stereoirregular oligonucleotides, the other monomers being natural *ribo*-configured DNA monomers. In contrast, the (almost) fully modified *xylo*-LNA (**ON18**) displayed very strong binding affinity towards DNA and RNA complements (ΔT_m +28 and +39 °C, respectively)[10] reflecting the importance of conformationally locked furanose configuration on the stability of the complexes formed in the case of stereoregular XNAs.

Table 2. Thermal[a] denaturation experiments of duplexes containing locked XNA monomers **L** and **N**.

		DNA target T_m (ΔT_m) [°C]	RNA target T_m (ΔT_m) [°C]
ON1	5′-d(GTGATATGC)	31 (ref.)[7]	29 (ref.)[7]
ON12	5′-d(GTGA**N**ATGC)	28 (–3)[7]	30 (+1)[7]
ON13	5′-d(G**N**GA**N**A**N**GC)	n.t.	19 (–10)[7]
ON14	5′-d(G$^{\alpha L}$T^{L}GANA$^{\alpha L}$T^{L}GC)	28 (–3)[7]	37 (+8)[7]
ON5	5′-T_{14}	30[7]/32[9,10] (ref.)	29[7]/28[9,10] (ref.)
ON15	5′-T_{10}	20[10] (ref.)	18[10] (ref.)
ON16	5′-T_7**L**T_6	19 (–13)[9]	24 (–4)[9]
ON17	5′-$T_5\mathbf{L}_4T_5$	n.t.[10]	n.t.[10]
ON18	5′-$\mathbf{L}_9$T	48 (+28)[10]	57 (+39)[10]
ON19	5′-T_7**N**T_6	23 (–7)[7]	26 (–3)[7]
ON20	5′-$\mathbf{X}_3(\mathbf{XN})_4\mathbf{X}_2$T	39 (+9)[7]	45 (+16)[7]

[a] Melting temperatures (T_m values) recorded in medium salt buffer using 1.5 μM concentrations of the two complementary strands. See footnote of Table 1 for further details; see Figure 1 for the structures of 2′-deoxy-XNA thymine monomer **X**, *xylo*-LNA thymine monomer **L**, 2′-amino-*xylo*-LNA thymine monomer **N** and α-L-LNA thymine monomer $^{\alpha L}\mathbf{T}^{\mathbf{L}}$.

The 2′-amino-*xylo*-LNA monomer **N** seems to stabilize the duplex when compared to other sugar-modified XNAs, and it displays many interesting features (Table 2). A single incorporation induces depression in the duplex stability (**ON12** relative to **ON1**, **ON19** relative to **ON5**), but even with three incorporations in a 9-mer, the destabilization against RNA target is limited to 10 °C (**ON13** relative to **ON1**).[7] Another interesting observation was made when this modification was incorporated together with two affinity-enhancing α-L-LNA ($^{\alpha L}\mathbf{T}^{\mathbf{L}}$)[9,13] monomers in a 9-mer strand with a centrally positioned monomer **N** (**ON14**). No depression of duplex stability was seen against the DNA target but an increase of +7 °C was observed against the RNA target (**ON14** relative to **ON12**).[7] Notably, monomer **N**, locked in an *N*-type, can also be combined with dXNA **X**, allowing fine tuning of the hybridization properties of XNAs. This was shown by the chimeric XNA **ON20**, which

against DNA and RNA targets displayed +2.3 and +1.8 °C increases in melting temperature per modification, respectively (**ON20** relative to **ON7**).[7] In contrast, monomer **F**, conformationally restricted (but not locked) in an *N*-type, showed no affinity enhancement in a similar chimeric sequence (**ON20** compared to **ON11**).[7] Finally, thermal denaturation studies of 9-mers **ON12**–**ON14** were carried out under different salt conditions (data not shown), but no significant changes in ΔT_m values were observed indicating that an affinity-enhancing electrostatic interaction between the 2′-amino group of monomer **N** and the phosphate backbone is unlikely, and that the increase in melting temperatures observed with this monomer is most likely due to entropically favoured duplex formation.

The XNA monomer **H** seems to stabilize the duplex when compared to its 2′-deoxy counterpart **X**, as it shows increased binding affinity towards the RNA target, both for one and three incorporations (Table 3, **ON21** relative to **ON2**, **ON29** relative to **ON6** and **ON22** relative to **ON3**). The furanose ring of monomer **H** is preorganized in an *N*-type conformation ($J_{1',2'}$ = 2 Hz in compound **12** as an example), which may contribute to this effect. Introduction of the 4′-*C*-hydroxymethyl group (monomers **M** and **K**) pointing towards the minor groove, has a negative effect on the duplex stability (with one or three incorporations) indicating the possibility of an unfavourable steric interaction involving the 4′-*C*-alkyl branch. Even the (almost) fully modified 4′-*C*-hydroxymethyl-XNA **ON31** was less stable compared to the reference duplex (**ON5**) or to the 2′-deoxy counterpart (**ON7**). Whereas no cooperative transition above 5 °C was observed between **ON31** and DNA, an association (T_m = 16 °C) was seen between **ON31** and RNA. As described earlier, recognition of DNA or RNA complements by fully modified homopyrimidine XNAs is due to triplex formation involving two XNA strands. This mode of binding is not possible with purine XNAs and it is reflected in the fact that no cooperative transition was detected above 5 °C with **ON40** against DNA or RNA targets.

A single incorporation of 4′-*C*-(*N*-methylpiperazinyl)-methyl-DNA in a 9-mer mixed sequence, when hybridized with a DNA target, was shown to increase the duplex stability by 4 °C.[22] The amino group, presumed to be partially protonated at physiological pH, is expected to contribute to overall stabilization of the duplex by interacting with the negatively charged phosphate backbone. However, a piperazinylmethyl group covalently attached to XNA (in monomers **P** and **Q**) has no stabilizing effect.

The $J_{1',2'}$ coupling constants of 5.6 Hz (**49a**), 5.5 Hz (**49b**), 5.3 Hz (**63**) and 5.1 Hz (**64**) show that these nucleosides are not adopting predominantly an *N*-type furanose conformation in solution but rather exist in an $N \rightleftharpoons S$ equilibrium. In addition to steric reasons, this can be another explanation for the poor hybridization properties of the corresponding monomers (**M**, **K**, **P** and **Q**).

Table 3.Thermal[a] denaturation experiments of duplexes containing XNA monomers **H**, **M**, **K**, **P** and **Q**.

		110 mM $[Na]^+$		710 mM $[Na]^+$	
		DNA target T_m (ΔT_m) [°C]	RNA target T_m (ΔT_m) [°C]	DNA target T_m (ΔT_m) [°C]	RNA target T_m (ΔT_m) [°C]
ON1	5′-d(GTGATATGC)	28[b], 30[c] (ref.)	26 (ref.)	32 (ref.)	31 (ref.)
ON21	5′-d(GTGA**H**ATGC)	27 (–3)[c]	30 (+4)		
ON22	5′-d(G**H**GA**H**A**H**GC)	n.t.	18 (–8)		
ON23	5′-d(GTGA**M**ATGC)	18 (–10)[b]	22 (–4)		
ON24	5′-d(G**M**GA**M**A**M**GC)	n.t.	n.t.		
ON25	5′-d(GTGA**P**ATGC)	19 (–9)[b]	20 (–6)	22 (–10)	26 (–5)
ON26	5′-d(G**P**GA**P**A**P**GC)	n.t.	n.t.	n.t.	n.t.
ON27	5′-d(GTGA**Q**ATGC)	19 (–9)[b]	22 (–4)	25 (–7)	29 (–2)
ON28	5′-d(G**Q**GA**Q**A**Q**GC)	n.t.	n.t.	n.t.	18 (–13)
ON5	5′-T_{14}	30 (ref.)	28 (ref.)	41 (ref.)	35 (ref.)
ON29	5′-T_7**H**T_6	18 (–12)	23 (–5)		
ON30	5′-T_7**M**T_6	19 (–11)	20 (–8)		
ON31	5′-$\mathbf{M}_{13}$T	n.t.	16 (–12)		
ON32	5′-T_7**P**T_6	21 (–9)	20 (–8)	31 (–10)	28 (–7)
ON33	5′-$\mathbf{X}_3(\mathbf{XP})_4\mathbf{X}_2$T	n.t.	n.t.	n.t.	n.t.
ON34	5′-T_7**Q**T_6	19 (–11)	22 (–6)	31 (–10)	31 (–4)
ON35	5′-d(GCATATCAC)	27 (ref.)	25 (ref.)	34	32
ON36	5′-d(GCAT**K**TCAC)	13 (–14)	16 (–9)	17 (–17)	22 (–10)
ON37	5′-d(GC**K**T**K**TC**K**C)	n.t.	n.t.	n.t.	n.t.
ON38	5′-A_{14}	33 (ref.)	n.t.	47	25
ON39	5′-A_7**K**A_6	23 (–10)	n.t.	37 (–10)	22 (–3)
ON40	5′-$\mathbf{K}_9$A	n.t.	n.t.	n.t.	n.t.

[a] Melting temperatures (T_m values) recorded in medium salt buffer or in high salt buffer (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.0 μM concentrations of the two complementary strands. See footnote of Table 1 for further details; see Figure 1 for the structures of 2′-deoxy-XNA thymine monomer **X**, XNA thymine monomer **H**, 4′-*C*-hydroxymethyl-XNA monomers **M** and **K** and 4′-*C*-piperazinylmethyl-XNA thymine monomers **P** and **Q**. [b],[c] Determined in different experimental series.

Conclusions

We have described here in detail the synthesis and hybridization properties of *xylo*-configured monomers, i.e., conformationally restricted 2′-deoxy-2′-fluoro-XNA (**F**) and XNA (**H**) monomers, conformationally locked 2′-amino-*xylo*-LNA monomer (**N**), 4′-*C*-hydroxymethyl-XNA monomers (**M** and **K**) and 4′-*C*-piperazinylmethyl-XNA monomers (**P** and **Q**). We have also described a significantly improved synthesis of *xylo*-LNA phosphoramidite **19** with an overall yield of 10% starting from commercially available 1,2:5,6-di-*O*-isopropylidine-α-D-allofuranose. Monomers **M**, **K**, **P** and **Q** were synthesised starting from furanose triol **43**, the key step being the stereoselective silylation of the triol **43** to yield furanose **44** in 93% yield. Various bicyclonucleosides and 4′α-branched *xylo*-configured nucleoside analogues can be synthesised starting from intermediate **44**. The furanose ring of monomers **F** and **H** adopts an *N*-type conformation, whereas the furanose ring in monomers **M**, **K**, **P** and **Q** likely exists in as N ⇌ S equilibrium. Thermal denaturation studies show that conformational restriction of XNA monomers leads to improved binding affinity as most clearly observed for XNAs **ON12**, **ON14**, **ON18** and **ON20** containing locked *xylo*-LNA monomers (**N** and **L**). Hybridization data of ninemer mixed-base sequences composed of a mixture of XNA monomers and DNA monomers revealed preferential hybridization towards RNA complements relative to DNA complements. Importantly, different monomeric nucleotides, including different XNA monomers and LNA monomers, can be combined, allowing fine-tuning of the hybridization properties of chimeric XNAs (e.g., **ON11**, **ON14** and **ON20**). It is clear from the results described earlier and herein that high-affinity targeting of homopurine DNA or RNA targets can be achieved via triplex formation using homopyrimidine XNAs; Mixed-sequence target sites, most efficiently RNA sequences, can be targeted efficiently by using chimera of XNA monomers and affinity-enhancing monomers, e.g., LNA or α-L-LNA monomers.

Experimental Section

General: Reactions were conducted under nitrogen when anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica-coated plates with fluorescence indicator (SiO_2-60, F-254) and visualizing under UV light and by spraying 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 and Silica gel 60 (particle size 0.015–0.040 mm, Merck) was used for Dry Column Vacuum Chromatography (DCVC).[33] 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 (unless otherwise stated). Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as internal standard for ^{1}H NMR and ^{13}C NMR, and relative to 85% H_3PO_4 as external standard for ^{31}P NMR spectroscopy. Coupling constants (*J* values) are given in Hertz. Assignments of NMR spectra, when given, are based on 2D spectra. Bicyclic compounds are named according to the von Baeyer nomenclature, whereas the atom numbering in the assignments follow the standard carbohydrate/nucleoside nomenclature. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode on a Kratos MS50TC spectrometer and MALDI-HRMS were recorded in positive ion mode on an IonSpec Fourier Transform mass spectrometer. 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.

1-*O*-Acetyl-2-deoxy-2-fluoro-3,5-di-*O*-benzoyl-α,β-D-xylofuranose (3): To a solution of 2-deoxy-3,5-di-*O*-benzoyl-2-fluoro-β-D-xylofuranoside (**2**)[24] (709 mg, 1.89 mmol) in a mixture of AcOH and Ac_2O (7.76 cm^3, 3.8:1, v/v) was added concd. H_2SO_4 (0.3 cm^3) and the resulting mixture was stirred at room temp. for 75 min. Then reaction mixture was slowly poured into ice-cold saturated aq. $NaHCO_3$ (500 cm^3) under vigorous stirring and extraction was performed by $CHCl_3$ (3 × 150 cm^3). The combined organic phase was washed successively with satd. aqueous $NaHCO_3$ (5 × 150 cm^3) and H_2O (2 × 100 cm^3), dried, evaporated to dryness and coevaporated with a mixture of toluene/EtOH (4 × 50 cm^3, 1:1, v/v) to give 0.74 g (97%) of anomeric mixture **3** as a yellow syrup which was used in next step without additional purification. R_f 0.32 (EtOAc/light petroleum, 20:80, v/v). ^{1}H NMR ($CDCl_3$): δ = 8.09–7.38 (m, 20 H), 6.51 (dd, *J* = 2.0 and 4.4 Hz, 1 H, 1-Hα), 6.40 (d, *J* = 12.4 Hz, 1 H, 1-Hβ), 5.97–5.78 (m, 2 H, 3-H), 5.38 (ddd, *J* = 4.8, 4.9 and 51.7 Hz, 1 H, 2-Hα), 5.20 (dd, *J* = 0.8 and 48.6 Hz, 1 H, 2-Hβ), 5.00–4.91 (m, 2 H, 4-H), 4.63–4.49 and 4.27–4.17 (m, 4 H, 5-H), 2.18 and 2.09 (3H each, 2s, 2 × $COCH_3$) ppm. ^{13}C NMR ($CDCl_3$, β-anomer): δ = 169.1, 166.0, 164.9, 133.9, 133.2, 129.8, 129.7, 129.4, 128.6, 128.3, 98.1 (d, *J* = 25.2 Hz), 97.1 (d, *J* 247.1), 79.9, 74.0 (d, *J* = 30.3 Hz), 62.6, 21.0 ppm. MALDI-HRMS: *m*/*z* 425.3532 ([M + Na]$^+$, $C_{21}H_{19}FO_7Na^+$ calcd. 425.3539).

1-(2-Deoxy-2-fluoro-β-D-xylofuranosyl)thymine (5b) and α-Anomer 5a: To a solution of anomeric mixture **3** (803 mg, 2.0 mmol) and bis(*O*-trimethylsilyl)thymine [obtained from thymine (277 mg, 2.2 mmol)] in anhydrous 1,2-dichloroethane (30 cm^3) was added TMS-triflate (1.2 cm^3, 1.47 g, 6.63 mmol) and the mixture was refluxed for 3 h. The reaction mixture was cooled to room temp., diluted with $CHCl_3$ (100 cm^3) and washed successively with saturated aq. $NaHCO_3$ (3 × 75 cm^3) and H_2O (100 cm^3). The organic layer was separated, dried and the solvents evaporated to dryness and the residue obtained was dissolved in a minimum volume of abs. EtOH and kept in the refrigirator for 3 h. The crystals formed were filtered, washed with cold EtOH and dried to give 373 mg (40%) of **4b**. R_f 0.71 ($CHCl_3$/EtOH, 20:1, v/v). The mother liquor was evaporated to dryness under reduced pressure. The residue (476 mg) was purified by column chromatography using a linear gradient [0–5% (v/v) MeOH in $CHCl_3$]. First eluted additional 88 mg (9%) of **4b** followed by 120 mg of an inseparable mixture of **4a** and **4b** and finally 220 mg (24%) of **4a** as a white foam. R_f 0.63 ($CHCl_3$/EtOH, 20:1, v/v). Compound **4b** (430 mg, 0.92 mmol) was stirred with saturated ammonia in MeOH (25 cm^3) at room temp. for 12 h. The reaction mixture was evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography using a linear gradient [0–10% (v/v) MeOH in $CHCl_3$] to give 200 mg (83%) of **5b** as a colourless syrup. R_f 0.35 ($CHCl_3$/EtOH, 90:10, v/v). ^{1}H NMR (CD_3OD): δ = 7.71 (d, *J* = 1.1 Hz, 1 H, 6-H), 6.02 (d, *J* = 21.4 Hz, 1 H, 1′-H), 4.95 (d, *J* = 49.4 Hz, 1 H, 2′-H), 4.35 (dd, *J* = 3.3 and 10.4 Hz, 1 H, 3′-H),

4.23 (ddd, J = 3.3, 6.0 and 8.8 Hz, 1 H, 4′-H), 4.00–3.86 (m, 2 H, 5′-H), 1.86 (d, J = 1.1 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR (CD_3OD): δ = 163.4, 152.3, 138.5, 110.9, 100.6 (d, J 184.9), 90.4 (d, J = 36.4 Hz), 85.0, 73.8 (d, J = 26.3 Hz), 60.7, 12.6 ppm. MALDI-HRMS: m/z 283.2153 ([M + Na]$^+$, $C_{10}H_{13}FN_2O_5Na^+$ calcd. 283.2088). Similar debenzoylation of 220 mg (0.47 mmol) of **4a** with saturated ammonia in MeOH (15 cm^3) and subsequent crystallization of the residue from EtOH afforded 91 mg (75%) of **5a** as colourless crystals. R_f 0.27 ($CHCl_3$/EtOH, 90:10, v/v). ^{1}H NMR (CD_3OD): δ = 7.45 (s, 1 H, 6-H), 6.24 (dd, J = 3.3 and 21.4 Hz, 1 H, 1′-H), 5.03 (dd, J = 3.3 and 51.1 Hz, 1 H, 2′-H), 4.67–4.64 (m, 2 H, 3′-H and 4′-H), 3.86 and 3.84 (1H each, 2s, 5′-H), 1.89 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR (CD_3OD): δ = 166.4, 152.1, 138.3 (d, J = 2.9 Hz), 110.6, 96.0 (d, J 191.2), 86.9 (d, J = 14.9 Hz), 84.2, 74.6 (d, J = 24.6 Hz), 61.4, 12.4 ppm. MALDI-HRMS: m/z 283.2033 ([M + Na]$^+$, $C_{10}H_{13}FN_2O_5Na^+$ calcd. 283.2088).

1-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-β-D-xylofuranosyl]-thymine (6): To a solution of nucleoside **5b** (299 mg, 1.15 mmol) in anhydrous pyridine (20 cm^3) was added dimethoxytrityl chloride (410 mg, 1.21 mmol) and the mixture was stirred at room temp. for 14 h. The reaction mixture was evaporated to dryness and the residue obtained was dissolved in $CHCl_3$ (100 cm^3) and washed successively with saturated aq. $NaHCO_3$ (3 × 50 cm^3) and H_2O (2 × 50 cm^3). The organic layer was separated, dried and the solvents evaporated to dryness under reduced pressure. The crude product obtained was purified by column chromatography using a linear gradient [0–5% (v/v) MeOH in $CHCl_3$] to give 584 mg (90%) of nucleoside **6** as a yellow foam. R_f 0.57 ($CHCl_3$/EtOH, 20:1, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.50–7.30 (m, 10 H), 6.86–6.83 (m, 4 H), 6.01 (d, J = 20.9 Hz, 1 H, 1′-H), 5.11 (d, J = 48.4 Hz, 1 H, 2′-H), 4.37–4.34 (m, 2 H, 3′-H and 4′-H), 3.78 (s, 6 H, 2 × OCH_3), 3.67–3.58 (m, 2 H, 5′-H), 1.72 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 164.1, 158.7, 150.6, 144.2, 137.1, 135.2 (d, J = 4.0 Hz), 130.0, 129.9, 128.0, 127.9, 127.0, 113.3, 110.0, 98.2 (d, J = 185.5 Hz), 90.0 (d, J = 38.4 Hz), 87.1, 81.9, 73.9 (d, J = 28.1 Hz), 61.7, 55.2, 12.4 ppm. MALDI-HRMS: m/z 585.5699 ([M + Na]$^+$, $C_{31}H_{31}FN_2O_7Na^+$ calcd. 585.5752).

1-{3-*O*-[2-Cyanoethoxy(diisopropylamino)phosphanyl]-2-*O*-deoxy-5-*O*-(4,4′-dimethoxytrityl)-2-fluoro-β-D-xylofuranosyl}thymine (7): 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (213 mg, 0.9 mmol) was added dropwise to a stirred solution of nucleoside **6** (338 mg, 0.6 mmol) and diisopropylethylamine (1.0 cm^3) in anhydrous CH_2Cl_2 (5.0 cm^3). After stirring for 30 min at room temp., the reaction mixture was diluted with EtOAc (50 cm^3). Washing was performed with saturated aq. $NaHCO_3$ (2 × 25 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue obtained was purified by column chromatography using a linear gradient [10–60% EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)] to give amidite **7** as a white foam (370 mg, 81%). R_f 0.73 and 0.58 (EtOAc/CH_2Cl_2/Et_3N, 50:50:5, v/v/v). ^{31}P NMR ($CDCl_3$): δ = 153.8, 151.2 ppm.

1-(2-*O*-Acetyl-3,5-*O*-isopropylidene-β-D-xylofuranosyl)thymine (9): To a solution of 1-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)thymine (**8**)[29] (261 mg, 0.88 mmol) in anhydrous pyridine (3.0 cm^3) was added Ac_2O (0.2 cm^3) and the resulting mixture was stirred at room temp. for 12 h. The reaction mixture was evaporated to dryness under reduced pressure and coevaporated with a mixture of toluene and EtOH (1:1, v/v, 6 × 25 cm^3) affording 270 mg (97%) of nucleoside **10** as white foam which was used in the next step without further purification. R_f 0.47 ($CHCl_3$/EtOH, 20:1, v/v). ^{1}H NMR ($CDCl_3$): δ = 8.84 (br. s, 1 H, NH), 7.87 (s, 1 H, 6-H), 6.01 (s, 1 H, 1′-H), 5.10 (s, 1 H, 2′-H), 4.31 (m, 1 H, 4′-H), 4.19 (br. s, 2 H, 5′-H), 4.03 (m, 1 H, 3′-H), 2.14 (s, 3 H, $COCH_3$), 1.95 (s, 3 H, 5-CH_3), 1.48 and 1.41 [2s, 3 H each, CH_3(isopropylidene)] ppm. ^{13}C NMR ($CDCl_3$): δ = 169.1, 163.8, 150.2, 136.6, 110.1, 98.2, 89.0, 81.7, 77.2, 73.8, 72.2, 59.9, 28.7, 20.7, 18.5, 12.5 ppm. MALDI-HRMS: m/z 363.3063 ([M + Na]$^+$, $C_{15}H_{20}N_2O_7Na^+$ calcd. 363.3183).

1-(2-*O*-Acetyl-β-D-xylofuranosyl)thymine (10): A solution of nucleoside **9** (251 mg, 0.74 mmol) in 80% AcOH was heated at 65 °C for 2 h and stirred at room temp. for another 12 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was coevaporated with a mixture of toluene and EtOH (1:1, v/v, 5 × 30 cm^3). The residue was purified by column chromatography [1–2% (v/v) EtOH in $CHCl_3$] to yield 185 mg (84%) of diol **10** as a colourless syrup. R_f 0.1 ($CHCl_3$/EtOH, 20:1, v/v). ^{1}H NMR ([D$_6$]DMSO): δ = 11.35 (br. s, 1 H), 7.68 (d, J = 1.0 Hz, 1 H), 5.85–5.82 (m, 2 H), 4.95–4.85 (m, 2 H), 4.20 (m, 1 H), 4.01 (m, 1 H), 3.77–3.64 (m, 2 H), 2.08 (s, 3 H), 1.77 (d, J = 1.0 Hz, 3 H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 169.4, 163.3, 150.3, 136.5, 109.2, 87.3, 83.0, 81.7, 72.0, 58.8, 20.5, 12.4 ppm. MALDI-HRMS: m/z 323.2456 ([M + Na]$^+$, $C_{12}H_{16}N_2O_7Na^+$ calcd. 323.2544).

1-[2-*O*-Acetyl-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl]thymine (11): To a solution of nucleoside **10** (108 mg, 0.36 mmol) in anhydrous pyridine (5 cm^3) was added dimethoxytrityl chloride (135 mg, 0.4 mmol) and the resulting mixture was stirred for 12 h at room temp. The reaction mixture was poured with vigorous stirring into ice-cold saturated aq. $NaHCO_3$ (100 cm^3) and was extracted with $CHCl_3$ (3 × 50 cm^3). The organic phase was dried and the solvents evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography ($CHCl_3$) to afford 196 mg (90%) of nucleoside **11** as a yellow syrup. R_f 0.39 ($CHCl_3$/EtOH, 20:1, v/v). ^{1}H NMR ($CDCl_3$): δ = 9.14 (br. s, 1 H, NH), 7.50–7.20 (m, 10 H), 6.85–6.82 (m, 4 H), 5.88 (d, J = 2.0 Hz, 1 H, 1′-H), 5.13 (m, 1 H, 2′-H), 4.22–4.20 (m, 2 H, 3′-H and 4′-H), 3.78 (s, 6 H, 2 × OCH_3), 3.61–3.51 (m, 2 H, 5′-H), 2.11 (s, 3 H, $COCH_3$), 1.80 (d, J = 0.7 Hz, 5-CH_3, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 169.9, 164.1, 158.6, 158.5, 150.4, 144.2, 137.1, 135.3, 130.0, 129.9, 129.1, 127.9, 127.7, 127.6, 127.0, 113.2, 113.1, 110.6, 89.5, 86.9, 82.1, 81.2, 74.5, 61.6, 55.2, 20.7, 12.3 ppm. MALDI-HRMS: m/z 625.6186 ([M + Na]$^+$, $C_{33}H_{34}N_2O_9Na^+$ calcd. 625.6208).

1-{2-*O*-Acetyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphanyl]-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl}thymine (12): Phosphitylation of nucleoside **11** (362 mg, 0.6 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (213 mg, 0.9 mmol) in the presence of diisopropylethylamine (1.0 cm^3) in anhydrous CH_2Cl_2 (5.0 cm^3), followed by work-up as described above for amidite **7** and purification by column chromatography using a linear gradient [25–100% EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)] afforded amidite **12** as a white foam (420 mg, 87%). R_f 0.67 and 0.52 (EtOAc/CH_2Cl_2/Et_3N, 50:50:5, v/v/v). ^{31}P NMR (CH_3CN): δ = 154.3, 149.9 ppm.

1-[2-*O*-Acetyl-3-*O*-benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*threo*-pentofuranosyl]thymine (15): To a solution of an anomeric mixture of 3-*O*-benzyl-1,2-di-*O*-acetyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α,β-L-*threo*-pentofuranose (**14**)[32] (2.04 g, 4.0 mmol) in acetonitrile (60 cm^3) was added thymine (0.57 g, 4.5 mmol) and *N,O*-bis(trimethylsilyl)acetamide (2.2 cm^3, 1.83 g, 9.0 mmol). The reaction mixture was refluxed for 40 min to give a clear solution and then cooled to room temp. TMS-triflate (2.17 cm^3, 2.67 g, 12.0 mmol) was added and the resulting mixture refluxed for 3 h. The reaction mixture was concen-

trated to dryness under reduced pressure and redissolved in $CHCl_3$ (500 cm^3), washed successively with saturated aq. $NaHCO_3$ (3 × 100 cm^3) and H_2O (100 cm^3), dried and then concentrated to dryness in vacuo. The residue obtained was purified by column chromatography ($CHCl_3$) and then crystallized from EtOH to give 1.89g (72%) of nucleoside **15**. R_f 0.21 ($CHCl_3$/EtOH, 20:1, v/v). ^1H NMR ($CDCl_3$): δ = 9.36 (s, 1 H, NH), 7.42–7.31 (m, 6 H), 6.29 (d, J = 3.6 Hz, 1 H, 1′-H), 5.36 (dd, J = 2.8 Hz and 3.6, 1 H, 2′-H), 4.78 (d, J = 11.3 Hz, 1 H), 4.66 (d, J = 11.3 Hz, 1 H), 4.55 (d, J = 11.2 Hz, 1 H), 4.42 (d, J = 11.2 Hz, 1 H), 4.35 (d, J = 10.7 Hz, 1 H), 4.26 (d, J = 10.7 Hz, 1 H), 4.25 (d, J = 2.8 Hz, 1 H, 3′-H), 3.05 and 3.03 (3 H each, 2s, 2 × SO_2CH_3), 2.14 (s, 3 H, $COCH_3$), 1.80 (d, J = 1.0 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 169.8, 163.5, 150.4, 135.8, 135.1, 128.7, 128.7, 128.4, 112.4, 87.6, 84.7, 81.1, 79.4, 73.1, 67.0, 65.4, 37.6, 20.6, 12.3 ppm. MALDI-HRMS: *m*/*z* 599.5180 ([M + Na]$^+$, $C_{22}H_{28}N_2O_{12}S_2Na^+$ calcd. 599.5209).

(1*S*,3*R*,4*R*,7*R*)-7-Benzyloxy-1-(hydroxymethyl)-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (16): To a solution of the nucleoside **15** (1.3 g, 2.25 mmol) in EtOH (60 cm^3) was added H_2O (6 cm^3) and NaOH pallets (0.5 g, 12.5 mmol). The reaction mixture was refluxed during 60 h, neutralized by adding solid CO_2 and the solvents evaporated to dryness. The residue obtained was purified by column chromatography using a linear gradient (0–10% (v/v) EtOH in $CHCl_3$) to yield 0.62 g (76%) of nucleoside **16** as a white foam. R_f 0.08 ($CHCl_3$/EtOH, 20:1, v/v). ^1H NMR ($CDCl_3$): δ = 9.57 (br. s, 1 H), 7.42–7.11 (m, 6 H), 5.70 (s, 1 H), 4.56 (d, J = 1.7 Hz, 1 H), 4.49 (d, J = 11.0 Hz, 1 H), 4.31 (d, J = 11.0 Hz, 1 H), 4.17 (d, J = 12.1 Hz, 1 H), 4.12–4.07 (m, 3 H), 3.94 (d, J = 12.1 Hz, 1 H), 1.60 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 164.5, 150.2, 136.9, 136.0, 128.5, 128.3, 127.7, 108.2, 88.9, 88.4, 79.9, 76.2, 73.0, 58.5, 12.1 ppm. MALDI-HRMS: *m*/*z* 383.3414 ([M + Na]$^+$, $C_{18}H_{20}N_2O_6Na^+$ calcd. 383.3510).

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-1-[(4,4′-dimethoxytrityl)oxymethyl]-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (17): To a solution of nucleoside **16** (0.6 g, 1.66 mmol) in anhydrous pyridine (20 cm^3) was added freshly crystallized DMTCl (0.62 g, 1.83 mmol) and the resulting mixture was stirred at room temp. for 12 h. The reaction mixture was evaporated to dryness under reduced pressure and dissolved in $CHCl_3$ (200 cm^3), washed successively with saturated aq. $NaHCO_3$ (3 × 75 cm^3), H_2O (100 cm^3) and brine (50 cm^3) and dried (Na_2SO_4). The organic phase was concentrated to dryness and the residue obtained was purified by column chromatography using a linear gradient [10–50% (v/v) EtOAc in light petroleum] to afford 1.05 g (95%) of nucleoside **17** as a white foam. R_f 0.1 (EtOAc/light petroleum, 60:40, v/v). All analytical data were identical to those previously reported.[30]

(1*R*,3*R*,4*R*,7*R*)-1-[(4,4′-Dimethoxytrityl)oxymethyl]-7-hydroxy-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (18): To a solution of nucleoside **17** (663 mg, 1.0 mmol) in a mixture of EtOAc and abs. EtOH (30 cm^3, 9:1, v/v) was added 10% $Pd(OH)_2$/C (500 mg). The mixture was stirred under hydrogen at 70 °C for 28 h. The catalyst was filtered off and washed with a mixture of EtOAc and EtOH (2 × 30 cm^3, 9:1, v/v). The combined filtrate was evaporated to dryness and the residue obtained was purified by column chromatography using a linear gradient (0–5% (v/v) MeOH in $CHCl_3$) to give 384 mg (67%) of nucleoside **18**. All analytical data were identical to those previously reported.[9]

(1*R*,3*R*,4*R*,7*R*)-7-[2-Cyanoethoxy(diisopropylamino)phosphanyloxy]-1-[(4,4′-dimethoxytrityl)oxymethyl]-3-(thymin-1-yl)-2,5-dioxabicyclo[2.2.1]heptane (19): Phosphitilation of nucleoside **18** (344 mg, 0.6 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (213 mg, 0.9 mmol) in the presence of diisopropylethylamine (1.0 cm^3) and anhydrous CH_2Cl_2 (5.0 cm^3), followed by work-up procedure as described above for amidite **7** and purification by column chromatography using a linear gradient [25–100% EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)] afforded amidite **19** as a white foam (381 mg, 82%) identical in all the respects with the sample synthesized previously.[9]

1-[3-*O*-Benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*threo*-pentofuranosyl]thymine (20): Nucleoside **15** (16.18 g, 28.1 mmol) was dissolved in ice-cold saturated methanolic ammonia (250 cm^3), where upon the stirred mixture was warmed to room temp. over a period of 45 min. The reaction mixture was evaporated to dryness in vacuo. The residue was purified by DCVC [90–100% (v/v) EtOAc in *n*-heptane] to give nucleoside **20** (14.0 g, 93%) as a white foam. R_f 0.37 (EtOAc/*n*-heptane, 90:10, v/v). ^1H NMR ([D$_6$]DMSO): δ = 11.41 (br. s, 1 H, NH), 7.62 (d, J = 1.1 Hz, 1 H, 6-H), 7.42–7.28 (m, 5 H), 6.09 (d, J = 5.3 Hz, 1 H, 2′-OH), 5.93 (d, J = 7.3 Hz, 1 H, 1′-H), 4.77 [d, J = 11.7 Hz, 1 H, CH_2Ph(a)], 4.69 [d, J = 11.7 Hz, 1 H, CH_2Ph(b)], 4.53 (d, J = 10.6 Hz, 1 H, 5′-Ha), 4.48 (ddd, J = 5.3, 5.5 and 6.8 Hz, 1 H, 2′-H), 4.41 (d, J = 10.6 Hz, 1 H, 5′′-Ha), 4.34 (d, J = 10.6 Hz, 1 H, 5′-Hb), 4.33 (d, J = 10.6 Hz, 1 H, 5′′-Hb), 4.22 (d, J = 5.5 Hz, 1 H, 3′-H), 3.24 (s, 3 H, SO_2CH_3), 3.17 (s, 3 H, SO_2CH_3), 1.76 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 163.6 (C-4), 150.9 (C-2), 137.7, 135.8 (C-6), 128.3, 127.8, 127.7, 110.4 (C-5), 85.4 (C-1′), 82.8 (C-3′), 80.7 (C-4′), 75.8 (C-2′), 72.4 (CH_2Ph), 69.2 and 68.6 (C-5′ and C-5′′), 36.7 and 36.6 (2 × SO_2CH_3), 12.0 (5-CH_3) ppm. ESI-MS: *m*/*z* 535.1 ([MH]$^+$, $C_{20}H_{27}N_2O_{11}S_2^+$ calcd. 535.1).

1-[3-*O*-Benzyl-2,5-di-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*threo*-pentofuranosyl]thymine (21): To a solution of nucleoside **20** (13.98 g, 26.2 mmol) in anhydrous pyridine (50 cm^3) at 0 °C, was added methanesulfonyl chloride (4.1 cm^3, 52.3 mmol). The reaction mixture was stirred for 4 h, then ethyl acetate (200 cm^3) was added and the reaction mixture was washed with saturated aq. $NaHCO_3$ (4 × 100 cm^3). The organic phase was dried (Na_2SO_4), filtered and the solvent removed in vacuo. The residue was purified by DCVC [90–100% (v/v) EtcOAc in *n*-heptane] to give nucleoside **21** (13.8 g, 86%) as a white foam. R_f 0.41 (EtOAc/*n*-heptane, 90:10, v/v); ^1H NMR ($CDCl_3$): δ = 9.39 (br. s, 1 H, NH), 7.40–7.28 (m, 6 H), 6.14 (d, J = 3.4 Hz, 1 H, 1′-H), 5.29 (t, J = 3.4 Hz, 1 H, 2′-H), 4.75 [d, J = 11.4 Hz, 1 H, CH_2Ph(a)], 4.65 [d, J = 11.4 Hz, 1 H, CH_2Ph(b)], 4.61 (d, J = 11.2 Hz, 1 H), 4.43 (d, J = 3.4 Hz, 1 H, 3′-H), 4.38 (d, J = 11.2 Hz, 1 H), 4.33 (d, J = 10.8 Hz, 1 H), 4.24 (d, J = 10.8 Hz, 1 H), 3.19 (s, 3 H, SO_2CH_3), 3.05 (s, 3 H, SO_2CH_3), 3.02 (s, 3 H, SO_2CH_3), 1.86 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.3 (C-4), 150.5 (C-2), 135.6, 134.6 (C-6), 128.7, 128.7, 128.3, 112.2 (C-5), 88.0 (C-1′), 85.1 (C-4′), 83.1 (C-2′), 80.9 (C-3′), 73.3 (CH_2Ph), 66.6 and 66.2 (C-5′ and C-5′′), 38.7, 37.6 and 37.5 (3 × SO_2CH_3), 12.2 (5-CH_3) ppm. ESI-MS: *m*/*z* 635.1 ([M + Na]$^+$, $C_{21}H_{28}N_2O_{13}S_3Na^+$ calcd. 635.1).

2,2′-Anhydro-1-[3-*O*-benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*erythro*-pentofuranosyl]thymine (22): To a solution of nucleoside **21** (12.72 g, 20.8 mmol) in anhydrous acetonitrile (150 cm^3) was added DBU (3.42 cm^3, 22.84 mmol) and the reaction mixture was stirred for 1.5 h during which extensive precipitation of nucleoside **22** occurred. The reaction mixture was cooled to –20 °C and filtered. The precipitate was washed several times with MeOH affording nucleoside **22** (9.57 g, 89%) as a white solid material. R_f 0.39 (MeOH/CH_2Cl_2,10:90, v/v). ^1H NMR ([D$_6$]DMSO): δ = 7.79 (d, J = 1.3 Hz, 1 H, 6-H), 7.45–7.32 (m, 5 H), 6.31 (d, J = 5.7 Hz, 1 H, 1′-H), 5.61 (t, J = 5.7 Hz, 1 H, 2′-H), 4.77 [d, J = 11.5 Hz, 1 H, CH_2Ph(a)], 4.68 [d, J = 11.5 Hz, 1 H, CH_2Ph(b)], 4.48 (d, J = 5.7 Hz, 1 H, 3′-H), 4.38 (d, J = 10.9 Hz, 1 H), 4.32 (d,

J = 10.9 Hz, 1 H), 4.23 (d, J = 11.5 Hz, 1 H), 4.05 (d, J = 11.5 Hz, 1 H), 3.25 (s, 3 H, SO_2CH_3), 3.06 (s, 3 H, SO_2CH_3), 1.79 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 171.3 (C-4), 159.7 (C-2), 137.0, 131.9 (C-6), 128.4, 128.1, 128.0, 117.1 (C-5), 89.4 (C-1′), 84.0 (C-4′), 80.1 (C-2′), 78.8 (C-3′), 72.8 (CH_2Ph), 68.9 and 67.6 (C-5′ and C-5′′), 37.0 and 36.9 (2×SO_2CH_3), 13.6 (5-CH_3) ppm. ESI-MS: m/z 517.1 ([MH]$^+$, calcd. 517.1). $C_{20}H_{24}N_2O_{10}S_2$: calcd. C 46.50, H 4.68, N 5.42; found C 46.27, H 4.54, N 5.43.

1-[3-*O*-Benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*erythro*-pentofuranosyl]thymine (23): Nucleoside **22** (8.87 g, 17.2 mmol) was dissolved in a mixture of aqueous H_2SO_4 (0.4 M, 300 cm^3) and acetone (300 cm^3) and refluxed for 14 h with stirring. After cooling to room temp. the reaction mixture was evaporated to approx. half the volume under reduced pressure and cooled to –20 °C, which resulted in the precipitation of nucleoside **23**. The white precipitate was filtered and washed thoroughly with H_2O affording nucleoside **23** (9.0 g, 98%) as a white solid material. R_f 0.51 (MeOH/CH_2Cl_2,10:90, v/v). ^{1}H NMR ($CDCl_3$): δ = 10.99 (br. s, 1 H, NH), 7.86 (d, J = 1.1 Hz, 1 H, 6-H), 7.43–7.32 (m, 5 H), 6.11 (d, J = 3.4 Hz, 1 H, 1′-H), 5.20 [d, J = 11.9 Hz, 1 H, CH_2Ph(a)], 5.11 (br. s, 1 H, 2′-H), 4.86 (d, J = 12.0 Hz, 1 H), 4.61 (d, J = 11.9 Hz, 1 H), 4.44 (d, J = 4.2 Hz, 1 H, 3′-H), 4.43 (d, J = 10.8 Hz, 1 H), 4.37 [d, J = 12.0 Hz, 1 H, CH_2Ph(b)], 4.27 (d, J = 10.8 Hz, 1 H), 3.08 (s, 3 H, SO_2CH_3), 2.89 (s, 3 H, SO_2CH_3), 1.95 (s, 1 H, 2′-OH), 1.83 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 166.1 (C-4), 150.4 (C-2), 139.4 (C-6), 136.7, 128.5, 128.3, 128.0, 108.5 (C-5), 87.1 (C-1′), 82.3 (C-4′), 77.8 (C-3′), 72.0 (CH_2Ph), 69.3 (C-2′), 68.5 and 67.8 (C-5′ and C-5′′), 37.8 and 37.3 (2×SO_2CH_3), 11.8 (5-CH_3) ppm. ESI-MS: m/z 535.1 ([MH]$^+$, calcd. 535.1). $C_{20}H_{26}N_2O_{11}S_2$·0.25H_2O: calcd. C 44.56, H 4.95, N 5.20; found C 44.43, H, 4.75, N 5.55.

1-[2-*C*-Azido-3-*O*-benzyl-2-deoxy-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*threo*-pentofuranosyl]thymine (24): Nucleoside **23** (5.11 g, 9.57 mmol) was dissolved in anhydrous CH_2Cl_2 (300 cm^3) and cooled to –78 °C. Anhydrous pyridine (7.70 cm^3, 95.6 mmol) and DMAP (4.67 g, 38.2 mmol) were added, followed by the dropwise addition of trifluoromethanesulfonic anhydride (1.77 cm^3, 10.5 mmol) over a period of 15 min. The stirred reaction mixture was warmed to 0 °C. After 6 h, additional trifluoromethanesulfonic anhydride (0.40 cm^3, 2.38 mmol) was added. The reaction was stirred for another hour and ice-cold saturated aq. $NaHCO_3$ (50 cm^3) was added. The organic phase was separated and washed successively with saturated aq. $NaHCO_3$ (2×100 cm^3), aqueous HCl (1 M, 3×100 cm^3) and saturated aq. $NaHCO_3$ (2×100 cm^3). The organic phase was dried with Na_2SO_4 and the solvents evaporated to dryness in vacuo. The residue was purified by DCVC [60–90% (v/v) EtOAc in *n*-heptane] to give an intermediate residue (5.1 g) as a light yellow foam. R_f 0.4 (MeOH/CH_2Cl_2, 5:95, v/v. ESI-MS: m/z 665.0). The yellow foam (5.1 g) was dissolved in anhydrous DMF (90 cm^3) and NaN_3 (550 mg, 8.46 mmol) was added and the reaction mixture stirred for 18 h. H_2O (200 cm^3) was added and the aqueous phase was extracted with a mixture of EtOAc and light petroleum (1:1, v/v, 5×100 cm^3). The combined organic phase was washed successively with saturated aq. $NaHCO_3$ (100 cm^3) and brine (100 cm^3), dried (Na_2SO_4) and the solvents evaporated to dryness under reduced pressure. The residue was purified by DCVC [60–80% (v/v) EtOAc in *n*-heptane] to give azide **24** (3.5 g) as white foam. R_f 0.65 (MeOH/CH_2Cl_2,10:90, v/v). NMR spectroscopic data revealed the compound to be contaminated with traces of DMF. ^{1}H NMR ([D$_6$]DMSO): δ = 11.51 (br. s, 1 H, NH), 7.65 (s, 1 H, 6-H), 7.44–7.31 (m, 5 H), 6.06 (d, J = 8.2 Hz, 1 H, 1′-H), 4.83 (t, J = 8.2 Hz, 1 H, 2′-H), 4.77 [d, J = 11.4 Hz, 1 H, CH_2Ph(a)], 4.70 [d, J = 11.4 Hz, 1 H, CH_2Ph(b)], 4.55 (d, J = 10.8 Hz, 1 H), 4.48 (d, J = 10.8 Hz, 1 H), 4.43–4.37 (m, 3 H), 3.27 (s, 3 H, SO_2CH_3), 3.19 (s, 3 H, SO_2CH_3), 1.79 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 163.5, 150.7, 137.1, 135.3, 128.4, 128.1, 127.9, 110.9, 82.9, 82.1, 81.1, 73.1, 69.1, 68.1, 65.1, 36.8, 12.1 ppm. ESI-MS: m/z 558.1 ([M – H]$^-$, $C_{20}H_{24}N_5O_{10}S_2^-$ calcd. 558.1).

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-1-(methylsulfonyloxymethyl)-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (25): To a solution of nucleoside **24** (1.74 g) in THF (90 cm^3) was added aq. NaOH (2.0 M, 31 cm^3, 62 mmol) and trimethylphosphane in THF (1.0 M, 6.2 cm^3, 6.2 mmol). After stirring the reaction mixture for 72 h, it was evaporated to approximately half volume in vacuo and brine (100 cm^3) was added. The aqueous phase was extracted with ethyl acetate (2×150 cm^3) and CH_2Cl_2 (6×150 cm^3). TLC analysis of the aqueous phase indicated the presence of product so it was evaporated to dryness in vacuo and the residue was extracted with MeOH (3×150 cm^3). The two combined organic phases were mixed and concentrated to dryness under reduced pressure. The residue was purified by DCVC [3–10% (v/v) MeOH in CH_2Cl_2] to give nucleoside **25** (1.1 g, 53% from **23**) as white foam. R_f 0.3 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ($CDCl_3$): δ = 9.10 (br. s, 1 H, NH), 7.36 (d, J = 1.1 Hz, 1 H, 6-H), 7.32–7.29 (m, 3 H), 7.14–7.11 (m, 2 H), 5.63 (s, 1 H, 1′-H), 4.68 (d, J = 11.5 Hz, 1 H, 5′-Ha), 4.64 (d, J = 11.5 Hz, 1 H, 5′-Hb), 4.42 [d, J = 11.0 Hz, 1 H, CH_2Ph(a)], 4.30 [d, J = 11.0 Hz, 1 H, CH_2Ph(b)], 4.08 (d, J = 1.7 Hz, 1 H, 3′-H), 3.91 (d, J = 1.7 Hz, 1 H, 2′-H), 3.22 (d, J = 10.6 Hz, 1 H, 5′′-Ha), 3.18 (d, J = 10.6 Hz, 1 H, 5′′-Hb), 3.08 (s, 3 H, SO_2CH_3), 1.80 (br. s, 1 H, NH), 1.62 (d, J = 1.1 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =163.8 (C-4), 150.2 (C-2), 136.4, 135.8, 128.5, 128.3, 127.9, 108.3 (C-5), 90.9 (C-1′), 86.9 (C-4′), 80.9 (C-3′), 72.7 (CH_2Ph), 65.4 (C-5′), 59.2 (C-2′), 50.5 (C-5′′), 37.7 (SO_2CH_3), 12.1 (5-CH_3) ppm. ESI-MS: m/z 438.1 ([MH]$^+$, $C_{19}H_{24}N_3O_7S^+$ calcd. 438.1).

(1*R*,3*R*,4*R*,7*R*)-1-(Benzoyloxymethyl)-7-benzyloxy-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (26): To a solution of nucleoside **25** (1.27 g, 2.90 mmol) in anhydrous DMF (300 cm^3) was added sodium benzoate (4.2 g, 29.0 mmol) and 15-crown-5 (2.18 cm^3, 14.5 mmol). The reaction mixture was stirred at 120 °C for 30 h, cooled to room temp. and H_2O (600 cm^3) was added. Extraction was performed with a mixture of EtOAc and *n*-heptane (1:1, v/v, 5×300 cm^3). The combined organic phase was washed with saturated aq. $NaHCO_3$ (2×200 cm^3), dried (Na_2SO_4) and the solvents evaporated to dryness under reduced pressure. An analytical sample was purified by DVDC [3–5% (v/v) MeOH in EtOAc] to give nucleoside **26** as a yellow viscous oil. R_f 0.52 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ($CDCl_3$): δ = 9.23 (br. s, 1 H, NH), 8.02 (dd, J = 1.4 and 8.4 Hz, 2 H, Bz) 7.59 (tt, J = 1.4 and 7.5 Hz, 1 H, Bz), 7.46–7.41 (m, 3 H, 6-H and Bz), 7.25–7.23 (m, 3 H, Bn), 7.12–7.08 (m, 2 H, Bn), 5.70 (s, 1 H, 1′-H), 4.85 (d, J = 12.3 Hz, 1 H, 5′-Ha), 4.76 (d, J = 12.3 Hz, 1 H, 5′-Hb), 4.47 [d, J = 11.2 Hz, 1 H, CH_2Ph(a)], 4.25 [d, J = 11.2 Hz, 1 H, CH_2Ph(b)], 4.11 (br. s, 1 H, 3′-H), 3.71 (br. s, 1 H, 2′-H), 3.32 (d, J = 10.7 Hz, 1 H, 5′′-Ha), 3.19 (d, J = 10.7 Hz, 1 H, 5′′-Hb), 1.57 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =165.9 (C-4), 150.0 (C-2), 136.7, 135.9, 133.4, 129.6, 128.5, 128.4, 128.2, 128.0, 108.1 (C-5), 87.3 (C-1′), 79.3 (C-3′), 72.8 (CH_2Ph), 60.7 (C-5′), 59.2 (C-2′), 50.9 (C-5′′), 12.1 (5-CH_3) ppm; the signals for COPh and C4′ could not be identified. ESI-MS: m/z 464.2 ([MH]$^+$, $C_{25}H_{26}N_3O_6$ calcd. 464.2). The major part of this product was used in the next step without purification by DCVC.

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-1-(hydroxymethyl)-3-(thymin-1-yl)-2-oxa-5-azabicyclo[2.2.1]heptane (27): The yellow viscous oil **26** (≈2.0 g) was dissolved in saturated methanolic NH_3 (100 cm^3) at

5 °C, where upon the mixture was warmed to room temp. and stirred for 18 h. The reaction mixture was evaporated in vacuo, and purified by DVDC [8–10% (v/v) MeOH in CH_2Cl_2] to give nucleoside **27** (0.5 g, 48% from **25**) as a white foam. R_f 0.23 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ([D$_6$]DMSO): δ = 11.17 (br. s, 1 H, NH), 7.42 (d, J = 1.3 Hz, 1 H, H-6), 7.31–7.24 (m, 3 H), 7.18–7.14 (m, 2 H), 5.47 (s, 1 H, 1′-H), 4.99 (br. s, 1 H, NH), 4.46 [d, J = 11.5 Hz, 1 H, CH_2Ph(a)], 4.28 [d, J = 11.5 Hz, 1 H, CH_2Ph(b)], 3.92 (d, J = 1.7 Hz, 1 H, 3′-H), 3.90 (d, J = 11.7 Hz, 1 H, 5′-Ha), 3.80 (d, J = 11.7 Hz, 1 H, 5′-Hb), 3.59 (d, J = 1.7 Hz, 1 H, 2′-H), 3.06 (d, J = 10.1 Hz, 1 H, 5′′-Ha), 2.77 (d, J = 10.1 Hz, 1 H, 5′′-Hb), 1.45 (d, J = 1.1 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 163.9 (C-4), 150.2 (C-2), 137.6, 136.9, 128.2, 127.5, 127.4, 106.1 (C-5), 89.8 (C-1′ and C-4′), 81.1 (C-3′), 71.4 (CH_2Ph), 58.9 and 57.8 (C-2′ and C-5′), 50.2 (C-5′′), 12.1 (5-CH_3) ppm. ESI-MS: m/z 360.2 ([MH]$^+$, $C_{18}H_{22}N_3O_5$ calcd. 360.2).

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-1-(hydroxymethyl)-3-(thymin-1-yl)-5-(trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptane (28): To a solution of nucleoside **27** (0.5 g, 1.39 mmol) in anhydrous MeOH (10 cm^3) was added DMAP (340 mg, 2.78 mmol) and ethyl trifluoroacetate (0.99 cm^3, 8.35 mmol). The resulting mixture was stirred for 16 h at room temp. The reaction mixture was evaporated to dryness in vacuo. The residue was purified by DVDC [80–100% (v/v) EtOAc in *n*-heptane] to give nucleoside **28** (0.59 g, 93%) as a white solid material. ^{1}H and ^{13}C NMR showed two conformations of the amide (rotamers) in 1:2 ratio. R_f 0.48 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ($CDCl_3$, major rotamer): δ = 8.68 (br. s, 1 H, NH), 7.30–7.27 (m, 4 H), 7.17–7.10 (m, 2 H), 5.58 (br. s, 1 H, 1′-H), 4.81 (br. s, 1 H, 2′-H), 4.55 [d, J = 11.2 Hz, 1 H, CH_2Ph(a)], 4.38 [d, J = 11.2 Hz, 1 H, CH_2Ph(b)], 4.22–4.11 (m, 2 H, 5′-H), 3.95 (d, J = 2.0 Hz, 1 H, 3′-H), 3.78 (d, J = 12.3 Hz, 1 H, 5′′-Ha), 3.59 (d, J = 12.3 Hz, 1 H 5′′-Hb), 2.32 (br. s, 1 H, 5′-OH), 1.61 (d, J = 1.1 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$, major rotamer): δ = 163.5 (C-4), 155.5 (q, $^2J_{C,F}$ = 38.2 Hz, $COCF_3$), 149.6 (C-2), 136.0, 135.2 (C-6), 128.9, 128.6, 128.0, 115.5 (q, $^1J_{C,F}$ 286.9, CF_3), 108.5 (C-5), 89.2 (C-4′), 88.3 (C-1′), 79.8 (C-3′), 73.5 (CH_2Ph), 60.8 (C-2′), 58.7 (C-5′), 52.7 (C-5′′), 12.2 (5-CH_3) ppm. ESI-MS: m/z 454.1 ([M – H]$^-$, calcd. 454.1). $C_{20}H_{20}F_3N_3O_6$: calcd. C 52.75, H 4.43, N 9.23; found C 52.38, H 4.39, N 8.88.

(1*R*,3*R*,4*R*,7*R*)-7-Hydroxy-1-(hydroxymethyl)-3-(thymin-1-yl)-5-(trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptane (29): To a solution of nucleoside **28** (494 mg, 1.08 mmol) in MeOH (125 cm^3) was added Pd/C (740 mg, 10% Pd on carbon) and the reaction was stirred under H_2 for 2 h. The reaction mixture was filtered through a plug of celite affording nucleoside **29** (382 mg, 96%) as a white solid material. R_f 0.41 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ([D$_6$]DMSO, major rotamer): δ = 11.25 (br. s, 1 H, NH), 7.53 (d, J = 1.3 Hz, 1 H, 6-H), 5.98 (br. s, 1 H, 3′-OH), 5.59 (s, 1 H, 1′-H), 5.14 (t, J = 5.7 Hz, 1 H, 5′-OH), 4.65 (br. s, 1 H, 2′-H), 4.18 (t, J = 2.4 Hz, 1 H, 3′-H), 3.92–3.84 (m, 2 H, 5′-H), 3.68 (d, J = 11.9 Hz, 1 H, 5′′-Ha), 3.42 (d, J = 11.9 Hz, 1 H, 5′′-Hb), 1.76 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ([D$_6$]DMSO, major rotamer): δ = 164.1 (C-4), 154.2 (q, $^2J_{C,F}$ = 37.4 Hz, $COCF_3$), 150.0 (C-2), 136.8 (C-6), 115.8 (q, $^1J_{C,F}$ = 287.3 Hz, CF_3), 106.1 (C-5), 89.2 (C-4′), 87.9 (C-1′), 72.3 (C-3′), 60.8 (C-2′), 57.2 (C-5′), 53.2 (C-5′′), 12.5 (5-CH_3) ppm. ESI-MS: m/z 364.1 ([M – H]$^-$; $C_{13}H_{13}F_3N_3O_6^-$ calcd. 364.1).

(1*R*,3*R*,4*R*,7*R*)-1-(4,4′-Dimethoxytrityloxymethyl)-7-hydroxy-3-(thymin-1-yl)-5-(trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptane (30): To a stirred solution of nucleoside **29** (104 mg, 0.28 mmol) in anhydrous pyridine (1.7 cm^3) at room temp. was added dimethoxytrityl chloride (145 mg, 0.43 mmol). After stirring for 7 h additional 4,4′-dimethoxytrityl chloride (19 mg, 0.06 mmol) was added, and the reaction mixture was stirred for further 11 h. EtOAc (20 cm^3) was added and the organic phase was washed successively with satd. aq. $NaHCO_3$ (3 × 15 cm^3) and brine (15 cm^3). The aqueous phase was extracted with EtOAc (15 cm^3) and the combined organic phase was dried (Na_2SO_4) and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue was subjected to DCVC [4–7% MeOH in CH_2Cl_2, containing 0.5% pyridine, v/v/v] to give nucleoside **30** (178 mg, 94%) as white solid material. R_f 0.62 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ([D$_6$]DMSO, major rotamer): δ = 11.28 (br. s, 1 H, NH), 7.48–7.23 (m, 10 H), 6.95–6.90 (m, 4 H), 5.97 (d, J = 2.9 Hz, 1 H, 3′-OH), 5.66 (s, 1 H, 1′-H), 4.70 (br. s, 1 H, 2′-H), 4.25 (t, J = 2.5 Hz, 1 H, 3′-H), 3.75 (s, 6 H, 2 × OCH_3), 3.69 (d, J = 11.5 Hz, 1 H, 5′-Ha), 3.64 (d, J = 11.5 Hz, 1 H, 5′-Hb), 3.60 (d, J = 10.6 Hz, 1 H, 5′′-Ha), 3.37 (d, J = 10.6 Hz, 1 H, 5′′-Hb), 1.70 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ([D$_6$]DMSO, major rotamer): δ = 163.9 (C-4), 158.1, 154.0 (q, $^2J_{C,F}$ 37.4, $COCF_3$), 149.8 (C-2), 144.4, 135.1, 134.9, 134.8, 129.7, 127.8, 126.1, 115.6 (q, $^1J_{C,F}$ = 287.6 Hz, CF_3), 113.2, 106.0 (C-5), 89.0 (C-4′), 87.9 (C-1′), 85.8 (CAr_3), 72.6 (C-3′), 62.3 (C-2′), 60.2 (C-5′), 54.9 (2 × OCH_3), 53.4 (C-5′′), 12.4 (5-CH_3) ppm. ESI-MS: m/z 666.2 ([M – H]$^-$, $C_{34}H_{31}F_3N_3O_8^-$ calcd. 666.2).

(1*R*,3*R*,4*R*,7*R*)-7-[2-Cyanoethoxy(diisopropylamino)phosphanyloxy]-1-(4,4′-dimethoxytrityloxymethyl)-3-(thymin-1-yl)-5-(trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptane (31): Nucleoside **30** (211 mg, 0.32 mmol) was co-evaporated with anhydrous acetonitrile (2 × 3 cm^3) under reduced pressure, dissolved in anhydrous CH_2Cl_2 (2 cm^3) and diisopropylethylamine (0.33 cm^3, 1.90 mmol) and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.21 cm^3, 0.95 mmol) were added. The mixture was stirred at room temp. for 8 h. MeOH (0.15 cm^3) and then EtOAc (20 cm^3) were added and washing was performed with satd. aq. $NaHCO_3$ (2 × 10 cm^3). The combined aqueous phase was extracted with EtOAc (10 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and the solvent evaporated to dryness in vacuo. The residue was purified by flash column chromatography [50% EtOAc in CH_2Cl_2, containing 1% Et_3N, v/v/v] to give nucleoside **31** (246 mg, 90%) as a white foam. R_f 0.46, 0.55 (MeOH/CH_2Cl_2, 5:95, v/v). ^{31}P NMR (500 MHz, $CDCl_3$): δ = 154.5, 153.7, 152.3, 152.0 ppm. ESI-MS: m/z 866.2 ([M – H]$^-$, $C_{43}H_{48}F_3N_5O_9P^+$ calcd. 866.3).

1-[2-*O*-Acetyl-3-*O*-benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*threo*-pentofuranosyl]-3-(benzyloxymethyl)thymine (32): Nucleoside **15** (5.02 g, 8.71 mmol) was dissolved in anhydrous CH_2Cl_2 (25 cm^3) at 0 °C with stirring. BOMCl (7.28 cm^3, 52.3 mmol) was added dropwise over a period of 5 min followed by addition of DIPEA (7.58 cm^3, 43.6 mmol) and the reaction mixture was warmed to room temp. and stirred for 44 h. The reaction mixture was diluted with CH_2Cl_2 (200 cm^3), washed successively with satd. aq. $NaHCO_3$ (2 × 150 cm^3) and half saturated aq. NH_4Cl (2 × 100 cm^3). The organic phase was dried (Na_2SO_4) and the solvents evaporated to dryness in vacuo. The residue was purified by DCVC [70–90% (v/v) EtOAc in *n*-heptane] to give nucleoside **32** (5.19 g, 86%) as a white foam, while 0.36 g (6%) of starting material **15** was recovered by elution with EtOAc. R_f 0.55 (MeOH/CH_2Cl_2, 5:95, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.41–7.28 (m, 11 H), 6.31 (d, J = 2.9 Hz, 1 H, 1′-H), 5.49 (d, J = 9.9 Hz, 1 H, BOM), 5.45 (d, J = 9.9 Hz, 1 H, BOM), 5.32 (dd, J = 2.2 and 2.9 Hz, 1 H, 2′-H), 4.79 (d, J = 11.2 Hz), 4.71–4.64 (m, 3 H), 4.57 (d, J = 11.2 Hz), 4.41 (d, J = 11.4 Hz), 4.35 (d, J = 10.7 Hz), 4.25 (d, J = 10.7 Hz), 4.22 (d, J = 2.2 Hz, 1 H, 3′-H), 3.02 (s, 6 H, 2 × SO_2CH_3), 2.20 (s, 3 H, $COCH_3$), 1.82 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 169.6, 162.9, 150.9, 137.8, 135.8, 133.7, 128.7, 128.6, 128.4, 128.2, 127.6, 111.6, 88.8, 85.1, 81.1, 79.7, 73.0, 72.1, 70.6,

66.8, 64.9, 37.8, 37.7, 20.7, 13.0 ppm. FAB-MS: *m*/*z* 697.1 ([MH]$^+$, $C_{30}H_{37}N_2O_{13}S_2^+$ calcd. 697.2).

1-[3-*O*-Benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*threo*-pentofuranosyl]-3-(benzyloxymethyl)thymine (33): A solution of nucleoside **32** (5.2 g, 7.46 mmol) in saturated methanolic NH_3 (250 cm^3) was stirred in an ice-bath for 15 min, then warmed to room temp. and subsequently evaporated to dryness under reduced pressure. The residue was purified by column chromatography [1% (v/v) MeOH in CH_2Cl_2] to give nucleoside **33** (4.6 g, 94%) as a white foam. R_f 0.50 (MeOH/CH_2Cl_2, 5:95, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.43–7.26 (m, 11 H), 5.78 (d, J = 4.4 Hz, 1 H, 1′-H), 5.43 and 5.42 (2s, 1 H each, BOM), 4.75–4.20 (m, 11 H), 3.04 (s, 3 H, SO_2CH_3), 2.99 (s, 3 H, SO_2CH_3), 1.91 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.0, 151.8, 137.7, 136.4, 133.7, 128.6, 128.5, 128.4, 128.2, 128.1, 127.7, 127.6, 126.9, 110.9, 91.6, 84.6, 83.1, 80.1, 72.9, 72.3, 70.5, 67.1, 37.6, 37.5, 12.9 ppm. ESI-MS: *m*/*z* 637.1 ([M – H_2O + H]$^+$, $C_{28}H_{33}N_2O_{11}S_2^+$ calcd. 637.2).

1-[3-*O*-Benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*erythro*-pentofuranosyl]-3-(benzyloxymethyl)thymine (35): Nucleoside **33** (46 mg, 0.07 mmol) was dissolved in CH_2Cl_2 (3 cm^3) and cooled to 0 °C. Pyridine (56 μL, 0.7 mmol) and DMAP (34 mg, 0.28 mmol) were added followed by the dropwise addition of trifluoromethanesulfonic anhydride (23 μL, 0.14 mmol) over a period of 2 min. After 4 h ice-cold satd. aq. $NaHCO_3$ (1.0 cm^3) was added. The organic phase was separated and washed with satd. aq. $NaHCO_3$ (2 × 1 cm^3), dried (Na_2SO_4) and the solvent evaporated to dryness in vacuo. The residue was purified by DCVC [55–75% (v/v) EtOAc in *n*-heptane] to give nucleoside **35** (33 mg, 72%) as a white foam. R_f 0.55 (MeOH/CH_2Cl_2, 5:95, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.59 (br. s, 1 H), 7.44–7.21 (m, 10 H), 6.12 (d, J = 2.9 Hz), 5.41 (s, 2 H), 4.92 (d, J = 11.7 Hz), 4.66 (s, 2 H), 4.64 (s, 2 H), 4.41–4.20 (m, 5 H), 3.15 (br. s, 1 H), 3.06 (s, 3 H), 3.03 (s, 3 H), 1.90 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.9, 151.3, 138.2, 137.2, 136.6, 129.2, 128.6, 128.0, 109.3, 85.9, 81.9, 79.8, 74.1, 72.4, 70.6, 69.6, 68.9, 68.4, 38.2, 37.9, 13.2 ppm. ESI-MS: *m*/*z* 655.2 ([MH]$^+$, $C_{28}H_{35}N_2O_{12}S_2^+$ calcd. 655.2).

1-[3-*O*-Benzyl-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-2-*O*-(trifluoromethylsulfonyl)-α-L-*erythro*-pentofuranosyl]-3-(benzyloxymethyl)thymine (36): Nucleoside **35** (19.0 g, 29.0 mmol) was dissolved in CH_2Cl_2 (1000 cm^3) and the resulting mixture was cooled to 0 °C. Pyridine (23.4 cm^3, 290.8 mmol) and DMAP (14.2 g, 116.3 mmol) were added followed by the dropwise addition of trifluoromethanesulfonic anhydride (9.78 cm^3, 58.2 mmol) over a period of 15 min. After 4 h ice-cold satd. aq. $NaHCO_3$ was added. The organic phase was separated and washed successively with satd. aq. $NaHCO_3$ (2 × 300 cm^3), 1 M aqueous HCl (3 × 300 cm^3) and satd. aq. $NaHCO_3$ (2 × 300 cm^3), dried (Na_2SO_4) and the solvents evaporated to dryness in vacuo. The residue was purified by DCVC [60–75% (v/v) EtOAc in *n*-heptane] to give triflated nucleoside **36** (16.0 g, 70%) as a white foam. R_f 0.65 (MeOH/CH_2Cl_2, 5:95, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.57–7.26 (m, 11 H), 6.25 (d, J = 2.6 Hz, 1 H, 1′-H), 5.46 (dd, J = 2.8 and 3.8 Hz, 2′-H), 5.42 (d, J = 9.8 Hz, 1 H, BOM), 5.39 (d, J = 9.8 Hz, 1 H, BOM), 4.80 [d, J = 11.2 Hz, 1 H, CH_2Ph(a)], 4.61–4.54 (m, 4 H), 4.47 [d, J = 11.2 Hz, 1 H, CH_2Ph(b)], 4.26 (d, J = 11.0 Hz), 4.21 (d, J = 12.2 Hz), 3.97 (d, J = 11.0 Hz), 2.96 (s, 3 H, SO_2CH_3), 2.91 (s, 3 H, SO_2CH_3), 1.97 (d, J = 1.3 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =162.8 (C-4), 150.6 (C-2), 137.5, 135.2, 133.7, 129.1, 128.9, 128.8, 128.2, 127.7, 118.0 (q, $^1J_{C,F}$ = 421.0 Hz, CF_3), 110.9 (C-5), 83.5 (C-1′), 82.4 (C-2′), 82.0 (C-4′), 77.1 (C-3′), 74.7 (BOM), 72.1 (CH_2Ph), 70.3 (BOM), 67.8 and 67.7 (C-5′ and C-5′′), 37.9 (SO_2CH_3), 37.6 (SO_2CH_3), 12.7 (5-CH_3) ppm. ESI-MS: *m*/*z* 787.1 ([MH]$^+$, $C_{29}H_{34}F_3N_2O_{14}S_3^+$ calcd. 787.1).

1-[2-*C*-Azido-3-*O*-benzyl-2-deoxy-5-*O*-(methylsulfonyl)-4-*C*-(methylsulfonyloxymethyl)-α-L-*threo*-pentofuranosyl]-3-(benzyloxymethyl)thymine (37): To a solution of nucleoside **36** (5.11 g, 6.49 mmol) in anhydrous DMF (100 cm^3) was added NaN_3 (465 mg, 7.14 mmol). The reaction mixture was stirred at room temp. for 18 h. H_2O (200 cm^3) was added and the aqueous phase was extracted with a mixture of EtOAc and light petroleum (1:1, v/v, 5 × 100 cm^3). The combined organic phase was washed successively with satd. aq. $NaHCO_3$ (100 cm^3) and brine (100 cm^3), dried (Na_2SO_4) and the solvents evaporated to dryness under reduced pressure. The residue was purified by DCVC [65–75% (v/v) EtOAc in *n*-heptane] to give azide **37** (3.66 g, 83%) as a white foam. R_f 0.62 (acetone/benzene, 20:80, v/v). IR (nujol): $\tilde{\nu}_{max}$ = 2114 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 7.42–7.23 (m, 11 H), 5.94 (d, J = 6.1 Hz, 1 H, 1′-H), 5. 49 (d, J = 9.7 Hz, 1 H, BOM), 5.47 (d, J = 9.7 Hz, 1 H, BOM), 4.75–4.63 (m, 4 H), 4.53 (d, J = 11.0 Hz), 4.38 (t, J = 6.1 Hz, 1 H, 2′-H), 4.35 (d, J = 11.0 Hz), 4.23 (d, J = 11.0 Hz), 4.19 (d, J = 6.1 Hz, 1 H, 3′-H), 4.17 (d, J = 11.2 Hz), 3.06 (s, 3 H, SO_2CH_3), 2.98 (s, 3 H, SO_2CH_3), 1.93 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =162.8, 150.9, 137.6, 135.7, 133.6, 128.8, 128.3, 128.2, 127.6, 111.7, 87.7, 83.4, 81.9, 74.0, 72.2, 70.6, 67.6, 67.4, 67.2, 37.7, 37.4, 12.9 ppm. FAB-MS: *m*/*z* 679.8 ([M]$^+$, $C_{28}H_{33}N_5O_{11}S_2^+$ calcd. 679.2).

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-3-[3-(benzyloxymethyl)thymin-1-yl]-1-(methylsulfonyloxymethyl)-2-oxa-5-azabicyclo[2.2.1]heptane (38): To a solution of triphenylphosphane (386 mg, 1.47 mmol) in anhydrous pyridine (50 cm^3) was added nucleoside **37** (505 mg, 0.74 mmol) and the resulting mixture was stirred at room temp. for 20 h. Concentrated aqueous NH_3 (50 cm^3) was added and the reaction mixture was stirred for additional 24 h. Extraction was performed with CH_2Cl_2 (3 × 70 cm^3) and the combined organic phase was successively washed with satd. aq. $NaHCO_3$ (2 × 50 cm^3) and satd. aq. NaCl (50 cm^3), dried (Na_2SO_4) and the solvents evaporated to dryness in vacuo. The residue was purified by DCVC [3–6% (v/v) MeOH in CH_2Cl_2] to give nucleoside **38** (410 mg, 99%) as a white foam. R_f 0.43 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.39–7.25 (m, 9 H), 7.10–7.06 (m, 2 H), 5.58 (s, 1 H, 1′-H), 5.49 (d, J = 9.6 Hz, 1 H, BOM), 5.44 (d, J = 9.6 Hz, 1 H, BOM), 4.70 (s, 2 H, BOM), 4.67 (d, J = 11.5 Hz, 1 H, 5′-Ha), 4.62 (d, J = 11.5 Hz, 1 H, 5′-Hb), 4.40 [d, J = 11.2 Hz, 1 H, CH_2Ph(a)], 4.25 [d, J = 11.2 Hz, 1 H, CH_2Ph(b)], 4.08 (d, J = 1.7 Hz, 1 H, 3′-H), 3.92 (d, J = 1.7 Hz, 1 H, 2′-H), 3.20 (d, J = 10.6 Hz, 1 H, 5′′-Ha), 3.16 (d, J = 10.6 Hz, 1 H, 5′′-Hb), 3.07 (s, 3 H, SO_2CH_3), 1.70 (br. s, 1 H, NH), 1.66 (d, J = 1.3 Hz, 3 H, 1 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =163.2 (C-4), 150.6 (C-2), 137.8, 135.8, 135.1 (C-6), 128.5, 128.4, 128.3, 128.1, 127.8, 127.5, 127.5, 107.6 (C-5), 91.3 (C-1′), 86.8 (C-4′), 80.9 (C-3′), 72.8 (CH_2Ph), 72.1 and 70.1 (BOM), 65.4 (C-5′), 59.2 (C-2′), 50.5 (C-5′′), 37.7 (SO_2CH_3), 12.9 (5-CH_3) ppm. FAB-MS: *m*/*z* 558.2 ([MH]$^+$, $C_{27}H_{32}N_3O_8S^+$ calcd. 558.2).

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-1-(benzoyloxymethyl)-3-[3-(benzyloxymethyl)thymin-1-yl]-2-oxa-5-azabicyclo[2.2.1]heptane (39): To a solution of nucleoside **38** (6.17 g, 11.1 mmol) in anhydrous DMF (300 cm^3) were added sodium benzoate (4.05 g, 28.6 mmol) and 15-crown-5 (0.63 g, 0.8 cm^3) and the resulting mixture was stirred at 120 °C for 24 h. The reaction mixture was cooled to room temp. and H_2O (600 cm^3) was added. Extraction was performed with a mixture of EtOAc and *n*-heptane (1:1, v/v, 5 × 300 cm^3) and the combined organic phase was washed with satd. aq. $NaHCO_3$ (2 × 200 cm^3), dried (Na_2SO_4) and the solvents evaporated to dryness under reduced pressure. The residue was purified by DCVC [3–5% (v/v) MeOH in EtOAc] to give nucleoside **39** (5.94 g, 92%) as a white foam. R_f 0.63 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR

($CDCl_3$, 400 MHz): δ = 8.02 (dd, J = 1.2 and 8.4 Hz, 2 H, Bz), 7.59 (tt, J = 1.2 and 7.5 Hz, 1 H, Bz), 7.45–7.36 (m, 6 H), 7.32 (t, J = 7.5 Hz, 1 H, Bz), 7.24–7.18 (m, 3 H), 7.05–7.01 (m, 2 H), 5.59 (s, 1 H, 1′-H), 5.50 (d, J = 9.7 Hz, 1 H, BOM), 5.46 (d, J = 9.7 Hz, 1 H, BOM), 4.84 (d, J = 12.3 Hz, 1 H, 5′-Ha), 4.75 (d, J = 12.3 Hz, 1 H, 5′-Hb), 4.72 (s, 2 H, BOM), 4.39 [d, J = 11.2 Hz, 1 H, CH_2Ph(a)], 4.25 [d, J = 11.2 Hz, 1 H, CH_2Ph(b)], 4.11 (d, J = 1.7 Hz, 1 H, 3′-H), 3.90 (d, J = 1.7 Hz, 1 H, 2′-H), 3.24 (d, J = 10.4 Hz, 1 H, 5′′-Ha), 3.19 (d, J = 10.4 Hz, 1 H, 5′′-Hb), 1.60 (br. s, 1 H, NH), 1.56 (d, J = 0.9 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 165.9 (COPh), 163.3 (C-4), 150.7 (C-2), 137.9 (BOM), 136.0, 135.5, 133.3, 129.6, 129.3, 128.4, 128.4, 128.2, 128.1, 127.8, 127.5, 107.4 (C-5), 91.2 (C-1′), 87.3 (C-4′), 81.2 (C-3′), 72.7 (CH_2Ph), 72.1 and 70.1 (BOM), 60.9 (C-5′), 59.2 (C-2′), 51.1 (C-5′′), 12.8 (5-CH_3) ppm. ESI-MS: m/z 584.3 ($[MH]^+$, $C_{33}H_{34}N_3O_7^+$ calcd. 584.2).

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-3-[3-(benzyloxymethyl)thymin-1-yl]-1-(hydroxymethyl)-2-oxa-5-azabicyclo[2.2.1]heptane (40): Nucleoside **39** (1.75 g, 3.0 mmol) was dissolved in saturated methanolic NH_3 (150 cm^3) at 5 °C, warmed to room temp. and stirred for 20 h. The reaction mixture was evaporated in vacuo, and purified by DCVC [8–10% (v/v) MeOH in EtOAc] to give nucleoside **40** (1.34, 93%) as a white foam. R_f 0.42 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR (C_5D_5N): δ = 7.76 (d, J = 1.1 Hz, 1 H, 6-H), 7.52–7.49 (m, 2 H), 7.35–7.21 (m, 8 H), 6.98 (br. s, 1 H, NH), 6.10 (s, 1 H, 1′-H), 5.74 (d, J = 9.5 Hz, 1 H, BOM), 5.72 (d, J = 9.5 Hz, 1 H, BOM), 4.96 (br. s, 1 H, 5′-OH), 4.91 (s, 2 H, BOM), 4.55 [d, J = 11.4 Hz, 1 H, CH_2Ph(a)], 4.50–4.41 (m, 2 H, 5′-H), 4.40 [d, J = 11.4 Hz, 1 H, CH_2Ph(b)], 4.35 (d, J = 1.8 Hz, 1 H, 3′-H), 4.22 (d, J = 1.8 Hz, 1 H, 2′-H), 3.66 (d, J = 10.3 Hz, 1 H, 5′′-Ha), 3.40 (d, J = 10.3 Hz, 1 H, 5′′-Hb), 1.73 (d, J = 1.1 Hz, 3 H, 5-CH_3) ppm. ^{13}C NMR (C_5D_5N): δ = 163.9 (C-4), 151.6 (C-2), 139.3, 138.1, 136.8, 128.9, 128.8, 128.4, 128.3, 128.1, 128.0, 107.1 (C-5), 91.9 and 91.4 (C-1′ and C-4′), 82.3 (C-3′), 72.8 (CH_2Ph), 72.4 and 70.9 (BOM), 60.4 (C-2′), 59.1 (C-5′), 51.7 (C-5′′), 13.4 (5-CH_3) ppm. ESI-MS: m/z 480.2 ($[MH]^+$, $C_{26}H_{30}N_3O_6^+$ calcd. 480.2).

(1*R*,3*R*,4*R*,7*R*)-7-Benzyloxy-3-[3-(benzyloxymethyl)thymin-1-yl]-1-(hydroxymethyl)-5-(trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptane (41): To a solution of nucleoside **40** (1.3 g, 2.71 mmol) in anhydrous MeOH (20 cm^3) were added DMAP (662 mg, 5.42 mmol) and ethyl trifluoroacetate (1.93 cm^3, 16.3 mmol). The reaction was stirred for 3 h at room temp., additional DMAP (166 mg, 1.36 mmol) and ethyl trifluoroacetate (0.32 cm^3, 2.71 mmol) were added, the reaction mixture was stirred for another 1 h and then evaporated to dryness in vacuo. The residue was purified by DCVC [70–100% (v/v) EtOAc in *n*-heptane] to give nucleoside **41** (1.21 g, 78%) as a white foam. ^{1}H and ^{13}C NMR showed it to be a mixture rotamers in the ratio 2:3. R_f 0.62 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ($CDCl_3$, major rotamer): δ = 7.40–7.27 (m, 9 H), 7.11–7.05 (m, 2 H), 5.61 (s, 1 H, 1′-H), 5.51 (d, J = 9.5 Hz, 1 H, BOM), 5.44 (d, J = 9.5 Hz, 1 H, BOM), 4.81 (br. s, 1 H, 2′-H), 4.72 (s, 2 H, BOM), 4.50 [d, J = 11.2 Hz, 1 H, CH_2Ph(a)], 4.33 [d, J = 11.2 Hz, 1 H, CH_2Ph(b)], 4.20–4.09 (m, 2 H, 5′-H), 3.98 (d, J = 1.8 Hz, 1 H, 3′-H), 3.75 (d, J = 12.1 Hz, 1 H, 5′′-Ha), 3.58 (d, J = 12.1 Hz, 1 H, 5′′-Hb), 2.19 (br. s, 1 H, 5′-OH), 1.71 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$, major rotamer): δ = 163.2 (C-4), 155.3 (q, $^2J_{C,F}$ = 37.7 Hz, $COCF_3$), 150.3 (C-2), 137.7, 135.4, 134.9, 128.7, 128.6, 128.5, 128.1, 128.0, 127.7, 127.6, 127.5, 115.5 (q, $^1J_{C,F}$ 287.0, CF_3), 107.8 (C-5), 89.3 and 88.2 (C-1′ and C-4′), 79.7 (C-3′), 73.5 (CH_2Ph), 72.1 and 70.1 (BOM), 58.7 and 58.4 (C-2′ and C-5′), 52.6 (C-5′′), 12.9 (5-CH_3) ppm. ESI-MS: m/z 576.2 ($[MH]^+$, $C_{28}H_{29}F_3N_3O_7^+$ calcd. 576.2).

(1*R*,3*R*,4*R*,7*R*)-7-Hydroxy-1-(hydroxymethyl)-3-(thymin-1-yl)-5-(trifluoroacetyl)-2-oxa-5-azabicyclo[2.2.1]heptane (29): Compound **41** (1.21 g, 2.1 mmol) was dissolved in anhydrous CH_2Cl_2 (30 cm^3) and stirred at –78 °C where upon BCl_3 (4.6 cm^3, 1 M in CH_2Cl_2) was added dropwise. The reaction mixture was slowly warmed to room temp. over a period of 2 h, cold MeOH (2 cm^3) was added dropwise followed by addition of $NaHCO_3$ (1.06 g) and the resulting mixture was evaporated to dryness under reduced pressure. The residue was purified by DCVC [9–10% (v/v) MeOH in CH_2Cl_2] to give a mixture of **29** and **42** as a white solid material. The mixture was stirred in H_2O (250 cm^3) for 24 h, then concentrated to dryness under reduced pressure affording **29** (699 mg, 91%) as a white solid material. Analytical data were identical to that listed above.

4-*C*-Hydroxymethyl-1,2-*O*-isopropylidene-α-D-xylofuranose (43):[35] Palladium hydroxide (20% on carbon, 10.0 g) was added to a solution of 3-*O*-benzyl-4-*C*-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-xylofuranose (**13**, 18.0 g) in methanol (100 cm^3). Ammonium formate (20.0 g) was added and the resulting mixture was refluxed for 16 h. The catalyst was removed by filteration through a short pad of silica gel and washed thoroughly with a hot mixture of $CHCl_3$ and MeOH (5:1, v/v, 250 cm^3). The filterate was concentrated to dryness under reduced pressure to yield furanoside **43** as a white solid material (11.4 g, 89%). R_f 0.19 (MeOH/CH_2Cl_2, 10:90, v/v).^{1}H NMR ([D$_6$]DMSO): δ = 5.84 (d, J = 4.0 Hz, 1-H), 5.30 (d, J = 5.2 Hz, 1 H, 3-OH), 4.65 (dd, J = 5.5 Hz and 5.9, OH), 4.51 (d, J = 4.2 Hz, 1 H, 2-H), 4.37 (dd, J = 5.3 Hz and 5.7, OH), 4.12 (d, J = 5.5 Hz, 1 H, 3-H), 3.55–3.39 (m, 4 H, 5-H and 5′-H), 1.45 and 1.24 [2s, 3 H each, CH_3(isopropylidene)] ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 111.3 [O*C*(CH_3)$_2$O], 104.0 (C-1), 90.2 (C-4), 88.3 (C-2), 75.7 (C-3), 61.1 (C-5 and C-5′), 27.1 and 26.6 [CH_3(isopropylidene)] ppm. MALDI-HRMS: m/z 243.0834 ($[M + Na]^+$, $C_9H_{16}O_6Na^+$ calcd. 243.0839).

5-*O*-(*tert*-Butyldiphenylsilyl)-4-*C*-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-xylofuranose (44): *tert*-Butyldiphenylsilyl chloride (3.96 g, 14.4 mmol) was added dropwise to a stirred suspension of diol **43** (3.52 g, 16 mmol) in a mixture of CH_2Cl_2 (20 cm^3) and triethylamine (10 cm^3). The reaction mixture was stirred for 12 h at room temp. Additional amount of *tert*-butyldiphenylsilyl chloride (880 mg, 3.2 mmol) was added and the resulting mixture was stirred for another 24 h. The reaction mixture was diluted with CH_2Cl_2 (200 cm^3) and washing was performed with saturated aq. $NaHCO_3$ (2 × 100 cm^3). The organic phase was dried (Na_2SO_4), filtered, concentrated and purified by column chromatography [35–40% (v/v), EtOAc in light petroleum] to give furanose **44** as a white solid material (6.82 g, 93%). R_f 0.35 (EtOAc/light petroleum, 40:60, v/v). ^{1}H NMR ([D$_6$]DMSO): δ = 7.75–7.69 (m, 4 H), 7.43–7.40 (m, 6 H), 5.90 (d, J = 4.4 Hz, 1 H, 1-H), 5.43 (d, J = 5.2 Hz, 1 H, 3-OH), 4.82 (dd, J = 5.1 Hz and 5.3, 5′-OH), 4.58 (dd, J = 1.6 Hz and 4.3, 2-H), 4.20 (dd, J = 1.3 Hz and 5.0, 3-H), 3.72 (s, 2 H, 5-H), 3.64 (dd, J = 5.3 and 10.7 Hz, 1 H, 5′-Ha), 3.53 (dd, J = 5.4 and 10.7 Hz, 1 H, 5′-Hb), 1.48 and 1.26 [2s, 3 H each, CH_3(isopropylidene)], 0.99 [s, 9 H, C(CH_3)$_3$] ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 135.2, 135.1, 133.1, 133.0, 129.7, 127.8, 111.5 [O*C*(CH_3)$_2$O], 104.2 (C-1), 90.3 (C-4), 88.4 (C-2), 75.7 (C-3), 63.6 (C-5), 61.1 (C-5′), 27.1 and 26.7 [CH_3(isopropylidene)], 26.5 [C(*C*H$_3$)$_3$], 18.8 [Si*C*(CH_3)$_3$] ppm. MALDI-HRMS: m/z 481.2001 ($[M + Na]^+$, $C_{25}H_{34}O_6SiNa^+$ calcd. 481.2017).

4-*C*-(Benzoyloxymethyl)-5-*O*-(*tert*-butyldiphenylsilyl)-1,2-*O*-isopropylidene-α-D-xylofuranose (45): Benzoyl chloride (2.1 cm^3, 18.1 mmol) was added dropwise to a stirred solution of furanose **44** (7.9 g, 17.2 mmol) in a mixture of pyridine (20 cm^3) and CH_2Cl_2 (50 cm^3), at 0 °C and the resulting mixture was stirred for 12 h at

room temp. Methanol (0.5 cm^3) was added and the resulting mixture was stirred for another 30 min. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in CH_2Cl_2 (200 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 100 cm^3). The organic phase was dried (Na_2SO_4), filtered, concentrated to dryness and purified by column chromatography [30% (v/v) EtOAc in light petroleum] to give furanose **45** as a white solid material (8.35 g, 86%). R_f 0.29 (EtOAc/light petroleum, 25:75, v/v). ^1H NMR ($CDCl_3$): δ = 7.89–7.86 (m, 2 H), 7.70–7.61 (m, 4 H), 7.53 (m, 1 H), 7.51–7.25 (m, 8 H), 6.06 (d, J = 3.8 Hz, 1 H, 1-H), 4.69 (d, J = 3.8 Hz, 1 H, 2-H), 4.56 (d, J = 11.6 Hz, 1 H, 5′-Ha), 4.38 (d, J = 11.6 Hz, 1 H, 5′-Hb), 4.37 (d, J = 5.1 Hz, 1 H, 3-H), 4.09 (d, J = 10.8 Hz, 1 H, 5-Ha), 3.95 (d, J = 10.5 Hz, 1 H, 5-Hb), 3.70 (d, J = 5.4 Hz, 1 H, 3-OH), 1.56 and 1.33 [2s, 3 H each, CH_3(isopropylidene)], 1.07 [s, 9 H, $C(CH_3)_3$] ppm. ^{13}C NMR ($CDCl_3$): δ =166.1 (COC_6H_5), 135.8, 135.7, 135.6, 133.1, 132.0, 131.9, 130.2, 129.9, 129.8, 128.4, 128.1, 128.0, 112.9 [$OC(CH_3)_2O$], 105.7 (C-1), 88.2 (C-4), 87.6 (C-2), 78.6 (C-3), 64.8 and 64.3 (C-5 and C-5′), 27.0 [CH_3(isopropylidene)], 26.9 [$C(CH_3)_3$], 26.3 [CH_3(isopropylidene)], 19.2 [$SiC(CH_3)_3$] ppm. MALDI-HRMS: m/z 585.2251 ([M + Na]$^+$, $C_{32}H_{38}O_7SiNa^+$ calcd. 585.2279).

4-*C*-(Benzoyloxymethyl)-3,5-di-*O*-(*tert*-butyldiphenylsilyl)-1,2-*O*-isopropylidene-α-D-xylofuranose (46): To a stirred solution of furanose **45** (12.0 g, 21.3 mmol) in a anhydrous DMF (25 cm^3) was added DMAP (244 mg, 2 mmol), imidazole (4.36 g, 64 mmol) and *tert*-butyldiphenylsilyl chloride (6.48 g, 23.3 mmol) and the resulting mixture was stirred 12 h at room temp. The reaction mixture was partitioned between 5% aqueous $KHSO_4$ (250 cm^3) and EtOAc (250 cm^3). Layers were separated and the aqueous layer was extracted with EtOAc (100 cm^3). The combined organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [5–6% (v/v) EtOAc in light petroleum] to give compound **46** as a white solid material (16.0 g, 93%). R_f 0.56 (EtOAc/light petroleum, 25:75, v/v). ^1H NMR ($CDCl_3$): δ = 7.77–7.73 (m, 4 H), 7.68–7.59 (m, 4 H), 7.54–7.49 (m, 3 H), 7.42–7.24 (m, 12 H), 7.19–7.14 (m, 2 H), 6.01 (d, J = 4.4 Hz, 1 H, 1-H), 4.63 (dd, J = 2.2 Hz and 4.4, 2-H), 4.53 (d, J = 2.1 Hz, 1 H, 3-H), 4.35 (d, J = 11.1 Hz, 1 H, 5′-Ha), 4.18 (d, J = 11.5 Hz, 1 H, 5′-Hb), 4.00 (d, J = 10.7 Hz, 1 H, 5-Ha), 3.84 (d, J = 11.0 Hz, 1 H, 5-Hb), 1.39 and 1.18 [2s, 3 H each, CH_3(isopropylidene)], 1.07 and 0.98 [2s, 9 H each, 2 × $C(CH_3)_3$] ppm. ^{13}C NMR ($CDCl_3$): δ =165.9 (COC_6H_5), 136.0, 135.9, 135.8, 135.7, 134.9, 133.1, 133.0, 132.9, 132.5, 130.0, 129.9, 129.8, 129.7, 128.3, 127.9, 127.8, 112.8 [$OC(CH_3)_2O$], 104.7 (C-1), 89.0 (C-4), 87.7 (C-2), 78.8 (C-3), 64.5 and 63.5 (C-5 and C-5′), 27.3 and 26.8 [CH_3(isopropylidene)], 27.1, 27.0 and 26.7 [$C(CH_3)_3$], 19.4 and 19.3 [$SiC(CH_3)_3$] ppm. MALDI-HRMS: m/z 823.3434 ([M + Na]$^+$, $C_{48}H_{56}O_7Si_2Na^+$ calcd. 823.3457).

1,2-Di-*O*-acetyl-4-*C*-(benzoyloxymethyl)-3,5-di-*O*-(*tert*-butyldiphenylsilyl)-α,β-D-xylofuranose (47): Concd. H_2SO_4 (0.12 cm^3, 2.4 μmol) was added, dropwise, to a stirred solution of furanose **46** (15.0 g, 18.7 mmol) in a mixture of acetic acid (160 cm^3) and acetic anhydride (16.0 cm^3, 170.1 mmol). After stirring for 4 h at room temp. the reaction mixture was poured carefully in an ice-water mixture (1000 cm^3) and stirred for another 30 min. Brine (250 cm^3) was added and extraction was performed with EtOAc (2 × 500 cm^3). The organic phase was dried (Na_2SO_4), filtered, concentrated to dryness under reduced pressure and coevoporated successively with absolute ethanol (2 × 100 cm^3) and toluene (2 × 20 cm^3). The residue was purified by column chromatography [15–20% (v/v) EtOAc in light petroleum] to give the anomeric mixture **47** as a white foam (13.8 g, 87%). R_f 0.41, 0.44 (EtOAc/light petroleum, 25:75, v/v). ^1H NMR ($CDCl_3$, major isomer): δ = 7.82 (d, J = 7.4 Hz, 2 H), 7.75–7.67 (m, 4 H), 7.61–7.21 (m, 17 H), 7.13–7.08 (m, 2 H), 6.10 (br. s, 1 H, 1-H), 5.16 (dd, J = 1.3 and 3.1 Hz, 1 H, 2-H), 4.47 (d, J = 3.0 Hz, 1 H, 3-H), 4.36 (d, J = 11.3 Hz, 1 H, 5′-Ha), 4.31 (d, J = 11.3 Hz, 1 H, 5′-Hb), 4.11 (d, J = 11.5 Hz, 1 H, 5-Ha), 4.03 (d, J = 11.6 Hz, 1 H, 5-Hb), 1.93 and 1.74 (2s, 3 H each, $COCH_3$), 1.08 and 0.91 [2s, 9 H each, 2 × $C(CH_3)_3$] ppm. ^{13}C NMR ($CDCl_3$, major isomer): δ = 169.5 and 169.2 (2 × $COCH_3$), 165.9 (COC_6H_5), 135.9, 135.8, 135.7, 133.3, 133.2, 133.1, 132.8, 131.7, 130.2, 130.1, 129.9, 129.8, 129.7, 128.4, 127.9, 127.8, 98.5 (C-1), 89.1 (C-4), 82.3 (C-2), 77.3 (C-3), 64.9 and 63.6 (C-5 and C-5′), 26.9 and 26.8 [$C(CH_3)_3$], 21.2 and 20.4 (2 × $COCH_3$), 19.3 and 19.2 [2 × $SiC(CH_3)_3$] ppm. MALDI-HRMS: m/z 867.3388 ([M + Na]$^+$, $C_{49}H_{56}O_9Si_2Na^+$ calcd. 867.3355). ^1H NMR ($CDCl_3$, minor isomer): δ = 7.81–7.73 (m, 6 H), 7.69–7.65 (m, 2 H), 7.61–7.58 (m, 2 H), 7.53 (m, 1 H), 7.47–7.29 (m, 12 H), 7.26–7.21 (m, 2 H), 6.41 (d, J = 4.9 Hz, 1 H, 1-H), 5.83 (dd, J = 4.9 and 9.2 Hz, 1 H, 2-H), 4.71 (d, J = 9.2 Hz, 1 H, 3-H), 4.29 (d, J = 12.1 Hz, 1 H, 5′-Ha), 4.04 (d, J = 10.7 Hz, 1 H, 5-Ha), 3.85 (d, J = 12.1 Hz, 1 H, 5′-Hb), 3.74 (d, J = 10.8 Hz, 1 H, 5-Hb), 1.69 and 1.49 (2s, 3 H each, 2 × $COCH_3$), 1.17 and 1.02 [2s, 9 H each, 2 × $C(CH_3)_3$] ppm.^{13}C NMR ($CDCl_3$, minor isomer): δ = 169.8 and 169.4 (2 × $COCH_3$), 165.7 (COC_6H_5), 136.1, 135.9, 135.8, 135.7, 135.6, 133.7, 133.1, 133.0, 132.5, 131.6, 130.2, 130.1, 130.0, 129.9, 129.8, 129.7, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.6, 92.6 (C-1), 84.3 (C-4), 76.3 (C-2), 73.2 (C-3), 63.6 and 63.5 (C-5 and C-5′), 27.0 and 26.8 [2 × $C(CH_3)_3$], 20.7 and 19.9 (2 × $COCH_3$), 19.3 and 19.2 [2 × $SiC(CH_3)_3$] ppm. MALDI-HRMS: m/z 867.3396 ([M + Na]$^+$, $C_{49}H_{56}O_9Si_2Na^+$ calcd. 867.3355).

1-[2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-3,5-di-*O*-(*tert*-butyldiphenylsilyl)-β-D-xylofuranosyl]thymine (48a): *N*,*O*-Bis(trimethylsilyl)acetamide (6.0 cm^3, 24.3 mmol) was added to a suspension of furanose **47** (7.2 g, 8.5 mmol) and thymine (1.33 g, 10.5 mmol) in anhydrous acetonitrile (100 cm^3). The reaction mixture was refluxed for 1 h whereupon the clear solution was cooled to room temp. TMS-triflate (2.1 cm^3, 11.6 mmol) was added dropwise and the resulting mixture was refluxed for 12 h. The reaction mixture was then cooled to room temp. and CH_2Cl_2 (200 cm^3) was added. Washing was performed with saturated aq. $NaHCO_3$ (2 × 100 cm^3) and the organic phase was dried (Na_2SO_4), filtered and the solvents evaporated to dryness under reduced pressure. The residue was purified by column chromatography [40–50% (v/v) EtOAc in light petroleum] to give nucleoside **48a** as a white solid material (7.06 g, 91%). R_f 0.27 (EtOAc/light petroleum, 40:60, v/v). ^1H NMR ($CDCl_3$): δ = 8.32 (br. s, 1 H, NH), 7.76–7.71 (m, 4 H),7.68–7.65 (m, 2 H), 7.61 (m, 1 H), 7.58–7.48 (m, 7 H), 7.47–7.32 (m, 7 H), 7.28–7.26 (m, 2 H), 7.23–7.11 (m, 3 H), 6.01 (d, J = 5.9 Hz, 1 H, 1′-H), 5.45 (dd, J = 5.5 and 5.6 Hz, 1 H, 2′-H), 4.54 (d, J = 5.9 Hz, 1 H, 3′-H), 4.38 (d, J = 11.5 Hz, 1 H, 5′′-Ha), 4.20 (d, J = 11.7 Hz, 1 H, 5′′-Hb), 4.19 (d, J = 11.4 Hz, 1 H, 5′-Ha), 3.90 (d, J = 11.7 Hz, 1 H, 5′-Hb), 1.63 (s, 3 H, $COCH_3$), 1.57 (d, J = 1.1 Hz, 3 H, 5-CH_3), 1.13 and 0.94 [2s, 9 H each, 2 × $C(CH_3)_3$] ppm. ^{13}C NMR ($CDCl_3$): δ =169.8 ($COCH_3$), 165.7 (COC_6H_5), 163.7 (C-4), 150.6 (C-2), 135.9, 135.8, 135.6, 135.5, 133.3, 132.9, 132.6, 132.5, 131.0, 130.4, 130.1, 130.0, 129.8, 129.4, 128.5, 128.1, 128.0, 127.8, 111.4 (C-5), 86.1 (C-4′), 85.3 (C-1′), 79.9 (C-2′), 75.4 (C-3′), 64.5 and 63.3 (C-5′ and C-5′′), 27.1 and 27.0 [2 × $C(CH_3)_3$], 20.3 ($COCH_3$), 19.5 and 19.2 [2 × $SiC(CH_3)_3$], 12.1 (5-CH_3) ppm. MALDI-HRMS: m/z 933.3530 ([M + Na]$^+$, $C_{52}H_{58}N_2O_9Si_2Na^+$ calcd. 933.3573).

9-[2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-3,5-di-*O*-(*tert*-butyldiphenylsilyl)-β-D-xylofuranosyl]-6-*N*-benzoyladenine (48b): $SnCl_4$ (2.2 cm^3, 18.8 mmol) was added to a suspension of furanose **47** (3.03 g, 3.58 mmol) and 6-*N*-benzoyladenine (1.62 g, 6.77 mmol) in anhydrous acetonitrile (30 cm^3). The reaction mixture was refluxed for

36 h, then cooled to room temp. and saturated aq. $NaHCO_3$ (100 cm^3) was added slowly. Extraction was performed with CH_2Cl_2 (2×100 cm^3), and the combined organic phase was washed with brine (2×100 cm^3), dried (Na_2SO_4) and the solvents evaporated to dryness under reduced pressure. The residue was purified by column chromatography [30–35% (v/v) EtOAc in light petroleum] to give nucleoside **48b** as a white solid material (2.43 g, 66%). R_f 0.41 (EtOAc/light petroleum, 50:50, v/v). ^1H NMR ($CDCl_3$): δ = 9.09 (s, 1 H, NH), 8.74 (s, 1 H, 8-H), 8.30 (s, 1 H, 2-H), 8.03 (d, J = 7.7 Hz, 2 H), 7.80 (d, J = 7.5 Hz, 2 H), 7.68 (d, J = 6.7 Hz, 4 H), 7.63–7.58 (m, 2 H), 7.55–7.50 (m, 4 H), 7.46–7.42 (m, 6 H), 7.38–7.30 (m, 5 H), 7.26–7.15 (m, 5 H), 6.14 (d, J = 5.1 Hz, 1 H, 1′-H), 5.88 (dd, J = 5.4 and 5.6 Hz, 1 H, 2′-H), 4.70 (d, J = 5.9 Hz, 1 H, 3′-H), 4.58 (d, J = 11.4 Hz, 1 H, 5′′-Ha), 4.36 (d, J = 11.6 Hz, 1 H, 5′-Ha), 4.25 (d, J = 11.4 Hz, 1 H, 5′′-Hb), 4.02 (d, J = 11.5 Hz, 1 H, 5′-Hb), 1.60 (s, 3 H, $COCH_3$), 1.11 and 0.92 (2s, 9 H each, 2×$C(CH_3)_3$) ppm. ^{13}C NMR ($CDCl_3$): δ =169.4 ($COCH_3$), 165.7 and 164.6 (2×COC_6H_5), 152.9, 151.6, 149.5, 141.4, 135.9, 135.7, 135.6, 135.5, 133.9, 133.2, 132.8, 132.7, 132.5, 130.9, 130.4, 130.1, 130.0, 129.8, 129.5, 128.9, 128.5, 128.0, 127.9, 127.8, 127.7, 122.8, 87.1 (C-4′), 85.3 (C-1′), 80.4 (C-2′), 75.7 (C-3′), 64.7 and 63.4 (C-5′ and C-5′′), 27.1 and 27.0 [2×$C(CH_3)_3$], 20.2 ($COCH_3$), 19.4 and 19.2 [2×$SiC(CH_3)_3$] ppm. MALDI-HRMS: m/z 1047.7639 ([M + Na]$^+$, $C_{59}H_{61}N_5O_8Si_2Na^+$ calcd. 1047.3051).

1-[2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-β-D-xylofuranosyl]thymine (49a): A solution of nucleoside **48a** (2.73 g, 3.0 mmol) and tetrabutylammonium fluoride (3.5 cm^3, 3.5 mmol, 1.0 M solution in THF) in THF (5 cm^3) was stirred for 2 h at room temp. Toluene (10 cm^3) was added and the mixture was then concentrated to approx. one third of the orginal volume under reduced pressure. The concentrated mixture was purified by column chromatography [4–5% (v/v) MeOH in CH_2Cl_2] to give nucleoside **49a** as a white solid material (1.21 g, 93%). R_f 0.28 (MeOH/CH_2Cl_2, 10:90, v/v). ^1H NMR ($CDCl_3$): δ = 9.56 (br. s, 1 H, NH), 8.05 (d, J = 7.1 Hz, 2 H), 7.58 (m, 1 H), 7.56–7.43 (m, 3 H), 5.89 (d, J = 5.6 Hz, 1 H, 1′-H), 5.45 (dd, J = 5.1 and 5.3 Hz, 1 H, 2′-H), 4.74 (br. s, 1 H, OH), 4.55 (d, J = 11.8 Hz, 1 H, 5′′-Ha), 4.44 (d, J = 4.4 Hz, 1 H, 3′-H), 4.36 (d, J = 11.9 Hz, 1 H, 5′′-Hb), 4.00 (d, J = 11.9 Hz, 1 H, 5′-Ha), 3.92 (d, J = 12.1 Hz, 1 H, 5′-Hb), 3.52 (br. s, 1 H, OH), 2.10 (s, 3 H, $COCH_3$), 1.91 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =171.3 ($COCH_3$), 166.4 (COC_6H_5), 164.1 (C-4), 150.8 (C-2), 137.8, 133.6, 129.8, 129.4, 128.7, 111.9 (C-5), 88.9 (C-1′), 86.6 (C-4′), 82.2 (C-2′), 76.9 (C-3′), 64.5 and 62.7 (C-5′ and C-5′′), 20.8 ($COCH_3$), 12.6 (5-CH_3) ppm. MALDI-HRMS: m/z 457.1197 ([M + Na]$^+$, $C_{20}H_{22}N_2O_9Na^+$ calcd. 457.1218).

9-[2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-β-D-xylofuranosyl]-6-*N*-benzoyladenine (49b): Tetrabutylammonium fluoride (1.75 cm^3, 1.75 mmol, 1.0 M solution in THF) was added to a solution of furanoside **48b** (1.78 g, 1.74 mmol) in THF (25 cm^3). The reaction mixture was stirred for 1 h at room temp. Toluene (25 cm^3) was added and the mixture concentrated to approx. one third of the original volume under reduced pressure. The concentrated mixture was purified by column chromatography [2–3%(v/v) MeOH in CH_2Cl_2) affording nucleoside **49b** as a white solid material (497 mg, 52%). R_f 0.42 (MeOH/CH_2Cl_2, 10:90, v/v). ^1H NMR ($CDCl_3$): δ = 9.21 (s, 1 H, NH), 8.70 (s, 1 H, 8-H), 8.02 (s, 1 H, 2-H), 7.97 (d, J = 7.1 Hz, 2 H), 7.96–7.92 (m, 2 H), 7.54–7.49 (m, 2 H), 7.44–7.36 (m, 4 H), 5.91 (d, J = 5.5 Hz, 1 H, 1′-H), 5.84 (dd, J = 5.3 and 5.6 Hz, 1 H, 2′-H), 5.55 (br. s, 1 H, OH), 5.37 (d, J = 10.8 Hz, 1 H, OH), 4.53–4.45 (m, 2 H, 3′-H and 5′′-Ha), 4.35 (d, J = 11.7 Hz, 1 H, 5′′-Hb), 3.95–3.92 (m, 2 H, 5′-H), 2.00 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR ($CDCl_3$): δ =170.2 ($COCH_3$), 166.3 and 164.7 (2×COC_6H_5), 152.2, 150.5, 150.4, 142.8, 133.6, 133.4, 133.1, 129.8, 129.5, 129.0, 128.7, 128.0, 124.2, 87.8 and 87.6 (C-1′ and C-4′), 81.7 (C-2′), 77.1 (C-3′), 64.2 and 63.1 (C-5′ and C-5′′), 20.7 ($COCH_3$) ppm. MALDI-HRMS: m/z 570.1585 ([M + Na]$^+$, $C_{27}H_{25}N_5O_8Na^+$ calcd. 570.1595).

1-[2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl]thymine (50a): A solution of nucleoside **49a** (350 mg, 0.81 mmol) and dimethoxytrityl chloride (328 mg, 0.97 mmol) in anhydrous pyridine (2 cm^3) was stirred for 12 h at room temp., CH_2Cl_2 (100 cm^3) was added and the resulting mixture was washed with saturated aq. $NaHCO_3$ (2×50 cm^3). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pressure and the residue was coevoporated with toluene (2×2.0 cm^3). The residue was purified by column chromatography [65–70% (v/v) EtOAc in light petroleum] to give nucleoside **50a** as a white solid material (482 mg, 81%). R_f 0.3 (MeOH/CH_2Cl_2, 5:95, v/v). ^1H NMR ($CDCl_3$): δ = 8.96 (s, 1 H, NH), 7.87 (d, J = 7.1 Hz, 2 H), 7.56 (m, 1 H), 7.47–7.22 (m, 12 H), 6.83–6.79 (m, 4 H), 6.04 (d, J = 4.6 Hz, 1 H, 1′-H), 5.44 (dd, J = 4.2 and 4.3 Hz, 1 H, 2′-H), 4.61 (d, J = 11.6 Hz, 1 H, 5′′-Ha), 4.42–4.38 (m, 2 H, 3′-H and 5′′-Hb), 3.76 (s, 3 H, OCH_3), 3.75 (s, 3 H, OCH_3), 3.69 (d, J = 7.4 Hz, 1 H, 3′-OH), 3.62 (d, J = 9.5 Hz, 1 H, 5′-Ha), 3.50 (d, J = 9.5 Hz, 1 H, 5′-Hb), 2.12 (s, 3 H, $COCH_3$), 1.62 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =170.7 ($COCH_3$), 166.1 (COC_6H_5), 163.7 (C-4), 158.9, 150.5 (C-2), 144.1, 137.1, 135.0, 134.9, 133.4, 130.2, 129.9, 129.4, 128.5, 128.2, 127.2, 113.4, 111.9 (C-5), 88.4 (C-1′), 87.6 and 86.7 (C-4′ and CAr_3), 82.1 (C-2′), 76.7 (C-3′), 64.1 and 62.0 (C-5′ and C-5′′), 55.3 (2×OCH_3), 20.8 ($COCH_3$), 12.1 (5-CH_3) ppm. MALDI-HRMS: m/z 759.2536 ([M + Na]$^+$, $C_{41}H_{40}N_2O_{11}Na^+$ calcd. 759.2524).

9-[2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl]-6-*N*-benzoyladenine (50b): Tritylation of nucleoside **49b** (364 mg, 0.66 mmol) was carried out with DMTCl (270 mg, 0.80 mmol) in the presence of pyridine (5.0 cm^3) as described above for **50a**. After the ususal work-up procedure, the residue obtained was purified by column chromatography [2% MeOH in CH_2Cl_2, containing 0.5% Et_3N (v/v/v)] to afford nucleoside **50b** as a white solid material (497 mg, 88%). R_f 0.28 (EtOAc/light petroleum, 75:25, v/v). ^1H NMR ($CDCl_3$): δ = 9.09 (s, 1 H, NH), 8.63 (s, 1 H, 8-H), 8.06 (s, 1 H, 2-H), 8.02 (d, J = 7.2 Hz, 2 H), 8.00–7.82 (m, 2 H), 7.61–7.49 (m, 5 H), 7.47–7.26 (m, 8 H), 7.19–7.16 (m, 2 H), 6.76–6.68 (m, 4 H), 6.09 (d, J = 2.0 Hz, 1 H, 1′-H), 5.46 (d, J = 1.9 Hz, 1 H, 2′-H), 4.97 (d, J = 11.4 Hz, 1 H, 5′′-Ha), 4.54 (d, J = 11.5 Hz, 1 H, 5′′-Hb), 4.48 (d, J = 11.0 Hz, 1 H, 3′-H), 3.73 and 3.72 (2s, 3 H each, 2×OCH_3), 3.63 (d, J = 9.6 Hz, 1 H, 5′-Ha), 3.58 (d, J = 9.4 Hz, 1 H, 5′-Hb), 2.16 (s, 3 H, $COCH_3$) ppm. ^{13}C NMR ($CDCl_3$): δ =170.3 ($COCH_3$), 166.3 and 164.4 (2×COC_6H_5), 158.5, 152.0, 150.3, 149.7, 144.6, 143.1, 135.7, 135.6, 133.5, 133.4, 133.1, 131.0, 130.2, 130.0, 129.6, 129.0, 128.9, 128.5, 127.9, 127.8, 126.8, 124.0, 113.1, 90.3 and 89.8 (C-1′ and C-4′), 86.6 (C-2′), 85.6 (CAr_3), 76.8 (C-3′), 62.3 and 60.7 (C-5′ and C-5′′), 55.2 (OCH_3), 20.8 ($COCH_3$) ppm. ESI-HRMS: m/z 850.3083 ([M + H]$^+$, $C_{48}H_{44}N_5O_{10}^+$ calcd. 850.3387).

1-{2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphanyl]-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl}thymine (51a): 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (170 mg, 0.72 mmol) was added dropwise to a stirred solution of the nucleoside **50a** (440 mg, 0.6 mmol) and diisopropylethylamine (1.0 cm^3) in anhydrous CH_2Cl_2 (5.0 cm^3). After stirring the resulting mixture was 12 h at room temp., the reaction mixture was diluted with EtOAc (50 cm^3). Washing was performed with saturated aq. $NaHCO_3$ (2×25 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and concentrated under reduced

pressure. The residue obtained was purified by column chromatography [45–60% EtOAc in *n*-hexane, containing 0.5% Et_3N (v/v/v)] to give amidite **51a** as a white foam (462 mg, 83%). R_f 0.46 and 0.40 (MeOH/CH_2Cl_2, 5:95, v/v). ^{31}P NMR ($CDCl_3$): δ = 154.1, 153.6 ppm. MALDI-HRMS: *m*/*z* 959.3622 ([M + Na]$^+$, $C_{50}H_{57}N_4O_{12}Na^+$ calcd. 959.3602).

9-{2-*O*-Acetyl-4-*C*-(benzoyloxymethyl)-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphanyl]-5-*O*-(4,4′-dimethoxytrityl)-β-D-xylofuranosyl}-6-*N*-benzoyladenine.(51b): Reaction of compound **50b** (462 mg, 0.54 mmol) with 2-cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (141 mg, 0.60 mmol) in the presence of diisopropylethylamine (1.0 cm^3) and anhydrous CH_2Cl_2 (5.0 cm^3) followed by workup procedure (as described above for compound **51a**) and column chromatography [50% EtOAc in *n*-hexane containing 0.5% Et_3N (v/v/v)] afforded phosphoramidite **51b** as a white foam (490 mg, 86%). R_f 0.23 and 0.31 (EtOAc/petroleum ether, 75:25, v/v). ^{31}P NMR ($CDCl_3$): δ = 154.3, 152.3 ppm.

3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-4-*C*-(hydroxymethyl)-1,2-*O*-isopropylidene-α-D-xylofuranose (52): A solution of furanose **46** (10.3 g 12.9 mmol) in saturated methanolic ammonia (200 cm^3) was stirred for 24 h at room temp. The solution was evaporated to dryness under reduced pressure and the residue was coevaporated with toluene (2 × 10 mL). The residue was purified by column chromatography [15% (v/v) EtOAc in light petroleum] to give furanoside **52** as a white solid material (8.1 g, 90%). R_f 0.60 (EtOAc/light petroleum, 25:75, v/v). ^1H NMR ($CDCl_3$): δ = 7.75–7.63 (m, 6 H), 7.59–7.56 (m, 2 H), 7.45–7.39 (m, 4 H), 7.36–7.24 (m, 8 H), 5.97 (d, *J* = 4.8 Hz, 1 H, 1-H), 4.78 (dd, *J* = 3.2 and 4.5 Hz, 1 H, 2-H), 4.43 (d, *J* = 3.1 Hz, 1 H, 3-H), 3.86 (d, *J* = 11.1 Hz, 1 H, 5-Ha), 3.64 (d, *J* = 11.1 Hz, 1 H, 5-Hb), 3.37 (dd, *J* = 5.8 and 11.8 Hz, 1 H, 5′-Ha), 3.19 (dd, *J* = 7.7 and 11.8 Hz, 1 H, 5′-Hb), 1.77 (dd, *J* = 5.9 and 7.6 Hz, 1 H, 5′-OH), 1.32 and 1.22 [2s, 3 H each, CH_3(isopropylidene)], 1.09 and 1.03 [2s, 9 H each, 2 × C(CH_3)$_3$] ppm. ^{13}C NMR ($CDCl_3$): δ = 136.1, 136.0, 135.9, 135.7, 133.1, 133.0, 132.9, 130.0, 129.9, 129.8, 127.9, 127.8, 127.7, 112.9 [O*C*(CH_3)$_2$O], 104.4 (C-1), 90.3 (C-4), 88.5 (C-2), 78.7 (C-3), 65.2 (C-5), 62.9 (C-5′), 27.6 and 27.5 [CH_3(isopropylidene)], 27.1 and 27.0 [C(*C*H_3)$_3$], 19.4 and 19.2 [Si*C*(CH_3)$_3$] ppm. MALDI-HRMS: *m*/*z* 719.3156 ([M + Na]$^+$, $C_{41}H_{52}O_6Si_2Na^+$ calcd. 719.3195).

3,5-Di-O-(*tert*-butyldiphenylsilyl)-1,2-*O*-isopropylidene-4-*C*-(*N*-methylpiperazinyl)methyl-α-D-xylofuranose (53a): To a stirred solution of furanose **52** (2.76 g, 3.96 mmol) in a mixture of pyridine (5 cm^3) and CH_2Cl_2 (30 cm^3), was added dropwise triflic anhydride (1.7 g, 6.03 mmol) at –30 °C over 30 min and the resulting mixture was stirred 12 h at room temp. The reaction mixture was washed with saturated. aq. $NaHCO_3$ (2 × 50 cm^3), dried (Na_2SO_4), filtered and the solvents evaporated to dryness under reduced pressure. The residue was coevaporated with toluene (2 × 25 cm^3) and then dissolved in THF (22 cm^3). *N*-methylpiperazine (3.9 g, 38.9 mmol) was added to the stirred solution and the resulting mixture was stirred at room temp. for 12 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was coevoporated with toluene (2 × 10 cm^3). The residue was purified by column chromatography [1% (v/v) MeOH in CH_2Cl_2] to give furanose **53a** as a pale yellow solid (2.86 g, 93%). R_f 0.73 (MeOH/CH_2Cl_2, 10:90, v/v). ^1H NMR ($CDCl_3$): δ = 7.69–7.56 (m, 8 H), 7.34–7.17 (m, 12 H), 6.00 (d, *J* = 4.8 Hz, 1 H, 1-H), 4.92 (dd, *J* = 4.4 and 4.6 Hz, 1 H, 2-H), 4.40 (d, *J* = 3.8 Hz, 1 H, 3-H), 3.78 (d, *J* = 10.9 Hz, 1 H, 5-Ha), 3.52 (d, *J* = 10.9 Hz, 1 H, 5-Hb), 2.48–1.95 [m, 13 H, 5′-H, NCH_3 and CH_2(piperazinyl)], 1.27 and 1.21 [2s, 3 H each, CH_3(isopropylidene)], 1.02 and 1.01 [2s, 9 H each, 2 × C(CH_3)$_3$] ppm. ^{13}C NMR ($CDCl_3$): δ =136.4, 136.3, 135.9, 135.7, 133.4, 133.3, 133.1, 133.0, 129.9, 129.8, 129.7, 129.6, 127.9, 127.8, 127.7, 127.6, 112.9 [O*C*(CH_3)$_2$O], 104.4 (C-1), 90.6 (C-4), 88.3 (C-2), 80.3 (C-3), 65.8 (C-5), 59.4 (C-5′), 55.1 and 54.5 [CH_2(piperazinyl)], 46.1 (NCH_3), 28.1 and 28.0 [CH_3(isopropylidene)], 27.2 and 27.0 [C(*C*H_3)$_3$], 19.5 and 19.1 [2 × Si*C*(CH_3)$_3$] ppm. MALDI-HRMS: *m*/*z* 802.4082 ([M + Na]$^+$, $C_{46}H_{62}N_2O_5Si_2Na^+$ calcd. 802.4090).

3,5-Di-O-(*tert*-butyldiphenylsilyl)-1,2-*O*-isopropylidene-4-*C*-piperazinylmethyl-α-D-xylofuranose (53b): Conversion into triflate of furanose **52** (8.58 g, 12.3 mmol) in a mixture of pyridine (15 cm^3) and CH_2Cl_2 (100 cm^3) with triflic anhydride (5.21 g, 18.5 mmol) was carried out as described above for **53a**. The crude residue obtained was dissolved in THF (34 cm^3), piperazine (10.5 g, 121.9 mmol) was added and the resulting mixture was stirred at room temp. for 12 h. The reaction mixture was concentrated to dryness under reduced pressure and the residue was coevoporated with toluene (2 × 25 cm^3). The residue was purified by column chromatography [10% (v/v) MeOH in CH_2Cl_2] to give furanose **53b** as a pale yellow solid material(8.6 g, 91%). R_f 0.35 (MeOH/CH_2Cl_2, 10:90, v/v). ^1H NMR ($CDCl_3$): δ = 7.69–7.55 (m, 8 H), 7.37–7.28 (m, 7 H), 7.27–7.18 (m, 5 H), 6.00 (d, *J* = 5.0 Hz, 1 H, 1-H), 4.92 (dd, *J* = 4.5 and 4.6 Hz, 1 H, 2-H), 4.39 (d, *J* = 4.0 Hz, 1 H, 3-H), 3.78 (d, *J* = 10.7 Hz, 1 H, 5-Ha), 3.51 (d, *J* = 10.7 Hz, 1 H, 5-Hb), 2.52–1.93 [m, 11 H, 5′-H, NH and CH_2(piperazinyl)], 1.28 and 1.22 [2s, 3 H each, CH_3(isopropylidene)], 1.02 and 1.01 [2s, 9 H each, 2 × C(CH_3)$_3$] ppm. ^{13}C NMR ($CDCl_3$): δ = 136.4, 136.3, 136.0, 135.7, 133.4 133.3, 133.1, 133.0, 129.9, 129.8, 129.7, 128.0, 127.9, 127.8, 127.7,127.6, 113.0 (O*C*(CH_3)$_2$O), 104.5 (C-1), 90.5 (C-4), 88.3 (C-2), 80.2 (C-3), 65.7 (C-5), 59.9 (C-5′), 55.5 and 45.6 [CH_2(piperazinyl)], 28.1 and 28.0 [CH_3(isopropylidene)], 27.2 and 27.0 [C(*C*H_3)$_3$], 19.5 and 19.1 [Si*C*(CH_3)$_3$] ppm. MALDI-HRMS: *m*/*z* 787.3904 ([M + Na]$^+$, $C_{45}H_{60}N_2O_5Si_2Na^+$ calcd. 787.3933).

3,5-Di-*O*-(*tert*-butyldiphenylsilyl)-1,2-*O*-isopropylidene-4-*C*-(*N*-trifluoroacetylpiperazinyl)methyl-α-D-xylofuranose (53c): Trifluoroacetic anhydride (2.34 cm^3, 16.3 mmol) was added to a stirred solution of furanose **53b** (6.36 g, 8.3 mmol) in a mixture of Et_3N (1.2 cm^3) and CH_2Cl_2 (40 cm^3) and the resulting mixture was stirred for 4 h at room temp. The reaction mixture was concentrated to dryness under reduced pressure and the residue was coevoparated with toluene (2 × 10 cm^3). The residue was purified by column chromatography [50% (v/v) EtOAc in light petroleum] to give furanose **53c** as a white solid (6.23 g, 87%). R_f 0.43 (EtOAc/light petroleum, 25:75, v/v). ^1H NMR ($CDCl_3$): δ = 7.69–7.54 (m, 8 H), 7.37–7.17 (m, 12 H), 6.01 (d, *J* = 4.8 Hz, 1 H, 1-H), 4.95 (dd, *J* = 4.3 and 4.7 Hz, 1 H, 2-H), 4.38 (d, *J* = 4.1 Hz, 1 H, 3-H), 3.78 (d, *J* = 11.0 Hz, 1 H, 5-Ha), 3.48 (d, *J* = 10.8 Hz, 1 H, 5-Hb), 3.22–3.07 [m, 4 H, CH_2(piperazinyl)], 2.32–1.95 [m, 6 H, 5′-H and CH_2(piperazinyl)], 1.29 and 1.24 [2s, 3 H each, CH_3(isopropylidene)], 1.03 and 1.02 [2s, 9 H each, 2 × C(CH_3)$_3$] ppm. ^{13}C NMR ($CDCl_3$): δ = 157.8 (q, $^2J_{C,F}$ = 38.9 Hz, $COCF_3$), 136.4, 136.2, 136.0, 135.7, 133.3, 133.2, 132.9, 130.1, 130.0, 129.9, 129.8, 127.9, 127.8, 127.7, 116.4 (q, $^1J_{C,F}$ = 288.0 Hz, CF_3), 113.1, 104.5, 90.4, 88.2, 80.2, 65.5, 58.8, 54.3, 53.7, 45.6, 43.2, 28.1, 28.0, 27.2, 27.0, 19.5, 19.1 ppm. MALDI-HRMS: *m*/*z* 883.3745 ([M + Na]$^+$, $C_{47}H_{59}F_3N_2O_6Si_2Na^+$ calcd. 883.3761).

1,2-*O*-Isopropylidene-4-*C*-(*N*-methylpiperazinyl)methyl-α-D-xylofuranose (54a): Tetrabutylammonium fluoride (7.6 cm^3, 7.6 mmol, 1.0 M solution in THF) was added to a solution of furanose **53a** (2.82 g, 3.62 mmol) in THF (20 cm^3) and the resulting mixture was stirred for 12 h at room temp. The reaction mixture was concentrated to dryness under reduced pressure and the residue was coevoporated with toluene (2 × 10 cm^3). The residue was purified by column chromatography [11% (v/v) MeOH in CH_2Cl_2] to give fu-

ranose **54a** as a pale yellow solid material (960 mg, 88%) which was crystallized from a 1:9 mixture of methanol and toluene. R_f 0.36 (MeOH/CH_2Cl_2, 15:85, v/v). ^{1}H NMR ($CDCl_3$): δ = 5.93 (d, J = 4.0 Hz, 1 H, 1-H), 4.60 (d, J = 4.1 Hz, 1 H, 2-H), 4.28 (s, 1 H, 3-H), 3.85 (s, 2 H, 5-H), 2.87 (d, J = 14.0 Hz, 1 H, 5′-Ha), 2.78–2.74 [m, 3 H, 5′-Hb and CH_2(piperazinyl)], 2.66 [br. s, 2 H, CH_2(piperazinyl)], 2.43 [br. s, 4 H, CH_2(piperazinyl)], 2.27 (s, 3 H, NCH_3), 1.56 and 1.31 [2s, 3 H each, CH_3(isopropylidene)] ppm. ^{13}C NMR ($CDCl_3$): δ = 112.4 [O*C*$(CH_3)_2$O], 105.3 (C-1), 88.3 (C-4), 87.6 (C-2), 79.2 (C-3), 66.2 (C-5), and 63.8 (C-5′), 55.4 and 55.2 [CH_2(piperazinyl)], 46.0 (NCH_3), 27.0 and 26.2 [CH_3(isopropylidene)] ppm. MALDI-HRMS: m/z 325.1736 ([M + Na]$^+$, $C_{14}H_{26}N_2O_5Na^+$ calcd. 325.1734).

1,2-*O*-Isopropylidene-4-*C*-(*N*-trifluoroacetylpiperazinyl)methyl-α-D-xylofuranose (54b): Tetrabutylammonium fluoride (17.7 cm^3, 17.7 mmol, 1.0 M solution in THF) was added to a solution of furanose **53c** (6.1 g, 7.1 mmol) in THF (50 cm^3). The reaction mixture was stirred for 12 h at room temp., concentrated to dryness under reduced pressure and the residue was coevoporated with toluene (2 × 15 cm^3). The residue obtained was purified by column chromatography [6% (v/v) MeOH in CH_2Cl_2] affording furanose **18b** as a white solid material (2.56 g). R_f 0.36 (MeOH/CH_2Cl_2, 10:90, v/v); NMR spectroscopic data revealed the compound to be contaminated with traces of tetrabutylammonium salts. ^{1}H NMR ($CDCl_3$): δ = 5.84 (d, J = 4.3 Hz, 1 H, 1-H), 4.48 (dd, J = 1.0 and 4.3 Hz, 1 H, 2-H), 4.05 (s, 1 H, 3-H), 3.77 (d, J = 12.0 Hz, 1 H, 5-Ha), 3.73 (d, J = 12.3 Hz, 1 H, 5-Hb), 3.53–3.46 [m, 4 H, CH_2(piperazinyl)], 2.77–2.68 [m, 2 H, CH_2(piperazinyl)], 2.64 (d, J = 14.0 Hz, 1 H, 5′-Ha), 2.57 (d, J = 14.1 Hz, 1 H, 5′-Hb), 2.50–2.39 [m, 2 H, CH_2(piperazinyl)], 1.43 and 1.19 [2s, 3 H each, CH_3(isopropylidene ppm. ^{13}C NMR ($CDCl_3$): δ =155.7 (q, J = 37.8 Hz), 116.5 (q, J = 287.9 Hz), 112.6, 105.3, 89.6, 87.9, 79.9, 65.0, 61.6, 54.7, 54.3, 45.9 (d, J = 2.7 Hz), 43.5, 27.1, 26.3 ppm. MALDI-HRMS: m/z 407.1391 ([M + Na]$^+$, $C_{15}H_{23}F_3N_2O_6Na^+$ calcd. 407.1400).

3,5-Di-*O*-benzoyl-1,2-*O*-isopropylidene-4-*C*-(*N*-methylpiperazinyl)-methyl-α-D-xylofuranose (55a): Benzoyl chloride (1.98 cm^3, 17.0 mmol) was added dropwise to a stirred solution of furanose **54a** (1.71 g, 5.65 mmol) in a mixture of pyridine (6 cm^3) and CH_2Cl_2 (15 cm^3) and the resulting mixture was stirred 12 h at room temp. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in CH_2Cl_2 (100 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The organic phase was dried (Na_2SO_4), filtered, concentrated and coevaporated with toluene (2 × 5 cm^3). The residue obtained was purified by column chromatography [5% (v/v) MeOH in CH_2Cl_2] to give the furanose **55a** (2.51 g, 87%) as a white solid material. R_f 0.52 (MeOH/CH_2Cl_2, 15:85, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.83–7.79 (m, 4 H), 7.43 (d, J = 7.4 Hz), 7.39 (d, J = 7.5 Hz), 7.32–7.21 (m, 4 H), 6.02 (d, J = 4.4 Hz, 1 H, 1-H), 5.57 (d, J = 2.0 Hz, 1 H, 3-H), 4.78 (dd, J = 1.9 Hz and 4.4, 2-H), 4.63 (d, J = 11.5 Hz, 1 H, 5-Ha), 4.35 (d, J = 11.7 Hz, 1 H, 5-Hb), 2.78–2.35 [m, 10 H, 5′-H and CH_2(piperazinyl)], 2.18 (s, 3 H, NCH_3), 1.64 and 1.32 [2s, 3 H each, CH_3-(isopropylidene)] ppm. ^{13}C NMR ($CDCl_3$): δ = 166.0 and 165.5 (2 × *C*OC_6H_5), 133.6, 133.3, 129.8, 129.6, 129.5, 129.1, 128.6, 128.5, 113.9 [O*C*$(CH_3)_2$O], 105.1 (C-1), 88.2 (C-4), 86.5 (C-2), 79.9 (C-3), 65.4 (C-5), 60.8 (C-5′), 55.2 and 54.8 [CH_2(piperazinyl)], 46.0 (NCH_3), 27.6 and 27.1 [CH_3(isopropylidene)] ppm. MALDI-HRMS: m/z 533.2261 ([M + Na]$^+$, $C_{28}H_{34}N_2O_7Na^+$ calcd. 533.2258).

3,5-Di-*O*-benzoyl-1,2-*O*-isopropylidene-4-*C*-(*N*-trifluoroacetylpiperazinyl)methyl-α-D-xylofuranose (55b): Dibenzoylation of furanose **54b** (2.80 g) with benzoyl chloride (2.57 cm^3, 22.1 mmol) in a mixture of pyridine (8 cm^3) and CH_2Cl_2 (20 cm^3) was carried out as described above for compound **55a**. The crude residue obtained after work-up procedure was purified by column chromatography [20% (v/v) EtOAc in light petroleum] to give furanose **55b** as a white solid material (3.84 g, 84% from **53c**). R_f 0.53 (MeOH/CH_2Cl_2, 5:95, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.85 (d, J = 7.7 Hz, 4 H), 7.51–7.27 (m, 6 H), 6.02 (d, J = 4.5 Hz, 1 H), 5.60 (br. s, 1 H), 4.81 (m, 1 H), 4.63 (d, J = 11.5 Hz, 1 H), 4.33 (d, J = 11.4 Hz, 1 H), 3.58–3.50 (m, 4 H), 2.87–2.74 (m, 4 H), 2.60–2.53 (m, 2 H), 1.61 (s, 3 H), 1.32 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 165.9, 165.5, 155.5 (d, J = 35.8 Hz), 133.8, 133.5, 133.4, 130.2, 129.8, 129.6, 129.5, 128.9, 128.7 128.6, 128.5, 116.5 (d, J = 287.9 Hz), 113.9, 105.2, 88.3, 86.2, 79.6, 64.7, 60.3, 54.7, 54.2, 46.0 (d, J = 3.0 Hz), 43.6, 27.5, 26.9 ppm. MALDI-HRMS: m/z 615.1917 ([M + Na]$^+$, $C_{29}H_{31}F_3N_2O_8Na^+$ calcd. 615.1925).

1,2-Di-*O*-acetyl-3,5-di-*O*-benzoyl-4-*C*-(*N*-methylpiperazinyl)methyl-α,β-D-xylofuranose (56a): A solution of furanose **55a** (2.5 g, 4.9 mmol) in 80% aqueous trifluoroacetic acid (25 cm^3) was stirred at room temp. for 3 h.The reaction mixture was concentrated to dryness under reduced pressure and the residue was coevaporated successively with absolute ethanol (2 × 20 cm^3), toluene (2 × 10 cm^3) and pyridine (2 × 5 cm^3). The yellow viscous oil obtained was dissolved in pyridine (10 cm^3), acetic anhydride (4.62 cm^3, 49 mmol) was added dropwise, and the resulting mixture was stirred for 12 h at room temp. The reaction mixture was concentrated to dryness and the residue was dissolved in CH_2Cl_2 (50 cm^3) and washing was performed with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The organic phase was dried (Na_2SO_4), filtered, concentrated to dryness under reduced pressure. The residue was purified by column chromatography [5% (v/v) MeOH in CH_2Cl_2] to give furanose **56a** (1:1 mixture of anomers, 2.46 g, 93%) as a white solid material. R_f 0.60 (MeOH/CH_2Cl_2, 15:85, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.86–7.80 (m, 8 H), 7.77–7.16 (m, 12 H), 6.41 [d, J = 4.6 Hz, 1 H, 1-H(A)], 6.16 [d, J = 2.1 Hz, 1 H, 1-H(B)], 5.84 [d, J = 4.0 Hz, 1 H, 3-H(B)], 5.79 [d, J = 8.6 Hz, 1 H, 3-H(A)], 5.65 [dd, J = 4.7 and 8.7 Hz, 1 H, 2-H(A)], 5.54 [dd, J = 2.1 and 3.9 Hz, 1 H, 2-H(B)], 4.49–4.37 (m, 4 H, 5-H), 2.89–2.10 [m, 20 H, 5′-H and CH_2(piperazinyl)], 2.13 and 2.10 (2s, 3 H each, NCH_3), 2.02 and 1.91 (2s, 6 H each, 4 × $COCH_3$) ppm. ^{13}C NMR ($CDCl_3$): δ =170.0, 169.8, 169.5, 165.9, 165.8, 165.4, 133.7, 133.6, 133.2, 133.1, 129.9, 129.8, 129.6, 129.5, 128.9, 128.7, 128.6, 128.5, 128.4, 98.6, 92.5, 88.9, 85.1, 81.2, 78.0, 76.4, 74.5, 64.5, 64.4, 63.1, 60.8, 55.5, 55.3, 54.9, 54.7, 46.1, 46.0, 21.2, 21.1, 20.9, 20.6 ppm. MALDI-HRMS: m/z 577.2165 ([M + Na]$^+$, $C_{29}H_{34}N_2O_9Na^+$ calcd. 577.2157).

1,2-Di-*O*-acetyl-3,5-di-*O*-benzoyl-4-*C*-(*N*-trifluoroacetylpiperazinyl)-methyl-α,β-D-xylofuranose (56b): Acetolysis of furanose **55b** (2.59 g, 4.37 mmol) was carried out with 80% trifluoroacetic acid (25 cm^3) following the procedure as described above for **56a**. The residue obtained after work-up procedure was acylated with acetic anhydride (4.15 cm^3, 44 mmol) in the presence of pyridine (10 cm^3) and the crude product obtained was purified by column chromatography [5% (v/v) MeOH in CH_2Cl_2] to give furanose **56b** (1:1 mixture of anomers, 2.46 g, 91%) as a white solid material. R_f 0.60 (MeOH/CH_2Cl_2, 5:95, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.88–7.81 (m, 8 H), 7.50–7.40 (m, 4 H), 7.36–7.19 (m, 8 H), 6.41 [d, J = 4.3 Hz, 1 H, 1-H(A)], 6.16 [br. s, 1 H, 1-H(B)], 5.84 [d, J = 4.1 Hz, 1 H, 3-H(B)], 5.80 [d, J = 8.8 Hz, 1 H, 3-H(A)], 5.67 [dd, J = 4.8 and 8.6 Hz, 1 H, 2-H(A)], 5.57 [m, 1 H, 2-H(B)], 4.55–4.43 (m, 4 H, 5-H), 3.60–3.50 (m, 8 H), 3.00 (d, J = 14.4 Hz), 2.86–2.61 (m, 9 H), 2.49–2.44 (m, 2 H), 2.08 (s, 6 H), 2.07 (s, 6 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.0, 169.7, 169.5, 169.4, 166.0, 165.8, 165.5, 155.7 (d, J = 35.5 Hz), 155.2 (d, J = 35.9 Hz), 133.9, 133.8, 133.6, 133.4,

133.3, 130.2, 129.9, 129.8, 129.6, 129.5, 129.4, 129.3, 128.6, 128.5, 116.5 (q, *J* 289.3), 98.5, 92.6, 88.6, 84.9, 81.0, 77.8, 76.1, 74.2, 64.1, 62.4, 60.5, 54.7, 54.5, 54.2, 54.1, 46.2, 46.0, 43.7, 43.5, 21.2, 21.1, 20.9, 20.5 ppm. MALDI-HRMS: *m*/*z* 659.1832 ([M + Na]$^+$, $C_{30}H_{31}F_3N_2O_{10}Na^+$ calcd. 659.1823).

1-[2-*O*-Acetyl-3,5-di-*O*-benzoyl-4-*C*-(*N*-methylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (57a): *N*,*O*-Bis(trimethylsilyl)acetamide (3.1 cm^3, 12.5 mmol) was added to a suspension of furanose **56a** (2.46 g, 4.57 mmol) and thymine (0.70 g, 5.55 mmol) in anhydrous acetonitrile (30 cm^3). The reaction mixture was refluxed for 1 h whereupon the clear solution was cooled to room temp. TMS-triflate (1.1 cm^3, 6.1 mmol) was added dropwise and the resulting mixture was refluxed for 3 h. The reaction mixture was then cooled to room temp. and diluted with CH_2Cl_2 (100 cm^3), washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The combined organic phase was dried (Na_2SO_4), filtered and the solvents evaporated to dryness under reduced pressure. The resulting yellow oil was purified by column chromatography [5% (v/v) MeOH in CH_2Cl_2] to give the nucleoside **57a** (2.15 g, 76%) as a white solid material. R_f 0.50 (MeOH/CH_2Cl_2, 15:85, v/v). ^{1}H NMR ($CDCl_3$): δ = 8.01–7.98 (m, 2 H), 7.88–7.84 (m, 2 H), 7.55 (m, 1 H), 7.49–7.42 (m, 3 H), 7.40–7.26 (m, 2 H), 7.21 (d, *J* = 1.1 Hz, 1 H, 6-H), 6.29 (d, *J* = 8.2 Hz, 1 H, 1′-H), 6.08 (d, *J* = 8.1 Hz, 1 H, 3′-H), 5.86 (t, *J* = 8.1 Hz, 1 H, 2′-H), 4.43 (d, *J* = 12.2 Hz, 1 H, 5′-Ha), 4.23 (d, *J* = 12.2 Hz, 1 H, 5′-Hb), 2.84–2.66 [m, 10 H, 5′′-H and CH_2(piperazinyl)], 2.40 (s, 3 H, NCH_3), 2.04 (s, 3 H, $COCH_3$), 1.46 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.4 (*C*OCH$_3$), 165.9 and 165.8 (2 × *C*OC$_6$H$_5$), 163.6 (C-4), 151.2 (C-2), 134.2, 134.0, 133.8, 129.8, 129.5, 129.3, 129.0, 128.7, 128.5, 112.4 (C-5), 84.6 (C-4′), 82.3 (C-1′), 75.9 and 75.5 (C-2′ and C-3′), 64.8 (C-5′), 60.4 (C-5′′), 55.5 and 54.5 [CH_2(piperazinyl)], 45.4 (NCH_3), 20.8(CO*C*H$_3$), 12.0 (5-CH_3) ppm. MALDI-HRMS: *m*/*z* 643.2373 ([M + Na]$^+$, $C_{32}H_{36}N_4O_9Na^+$ calcd. 643.2375).

1-[2-*O*-Acetyl-3,5-di-*O*-benzoyl-4-*C*-(*N*-trifluoroacetylpiperazinyl)-methyl-β-D-xylofuranosyl]thymine (57b): *N*,*O*-Bis(trimethylsilyl) acetamide (2.6 cm^3, 10.3 mmol) was added to a suspension of furanose **56b** (2.29 g, 3.69 mmol) and thymine (0.57 g, 4.52 mmol) in anhydrous acetonitrile (30 cm^3). The reaction mixture was refluxed for 1 h whereupon the clear solution was cooled to room temp. TMS-triflate (0.84 cm^3, 4.66 mmol) was added dropwise and the mixture was refluxed for 3 h. After work-up procedure, as described above for **57a**, the resulting yellow oil was purified by column chromatography [2% (v/v) MeOH in CH_2Cl_2] to give the nucleoside **57b** (2.15 g, 83%) as a white solid material. R_f 0.44 (MeOH/CH_2Cl_2, 10:90, v/v). ^{1}H NMR ($CDCl_3$): δ = 9.08 (br. s, 1 H, NH), 8.08–8.05 (m, 2 H), 7.96–7.93 (m, 2 H), 7.66–7.58 (m, 2 H), 7.56–7.48 (m, 2 H), 7.40–7.35 (m, 2 H), 7.26 (d, *J* = 1.2 Hz, 1 H, 6-H), 6.38 (d, *J* = 8.3 Hz, 1 H, 1′-H), 6.16 (d, *J* = 8.1 Hz, 1 H, 3′-H), 5.97 (dd, *J* = 8.2 and 8.3 Hz, 1 H, 2′-H), 4.51 (d, *J* = 12.2 Hz, 1 H, 5′-Ha), 4.29 (d, *J* = 12.4 Hz, 1 H, 5′-Hb), 3.93–3.73 [m, 4 H, CH_2(piperazinyl)], 2.95–2.82 [m, 5 H, 5′′-Ha and CH_2(piperazinyl)], 2.78 (d, *J* = 14.5 Hz, 1 H, 5′′-Hb), 2.12 (s, 3 H, $COCH_3$), 1.55 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 170.4, 166.1, 165.9, 163.4, 155.5 (q, *J* = 35.9 Hz), 151.0, 134.2, 134.1, 134.0, 129.9, 129.8, 129.5, 129.4, 129.3, 129.1, 128.8, 128.7, 128.3, 116.6 (q, *J* = 288.5 Hz), 112.5, 84.4, 82.2, 75.8, 75.0, 64.6, 60.4, 55.2, 54.7, 46.4, 43.9, 20.8, 12.1 ppm. MALDI-HRMS: *m*/*z* 725.2035([M + Na]$^+$, $C_{33}H_{33}F_3N_4O_{10}Na^+$ calcd. 725.2041).

1-[3,5-Di-*O*-benzoyl-4-*C*-(*N*-methylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (58): A solution of nucleoside **57a** (2.08 g, 3.35 mmol) in 10% saturated methanolic ammonia (80 cm^3) was stirred for 1 h at room temp. The solution was evaporated to dryness under reduced pressure. The residue was coevoporated with toluene (2 × 10 cm^3) and purified by column chromatography [7–8% (v/v) MeOH in CH_2Cl_2] to give unreacted nucleoside **57a** (620 mg, 30%), while [10% (v/v) MeOH in CH_2Cl_2] afforded the desired nucleoside **58** (1.1 g, 57%) as a white solid material. R_f 0.41 (MeOH/CH_2Cl_2, 15:85, v/v). ^{1}H NMR ($CDCl_3$/CD_3OD, 2:1): δ = 7.90–7.84 (m, 4 H), 7.56–7.27 (m, 7 H), 6.06 (d, *J* = 6.6 Hz, 1 H), 5.80 (d, *J* = 6.5 Hz, 1 H), 4.52 (dd, *J* = 6.5 and 6.6 Hz), 4.38 (s, 2 H), 4.04 (br. s, 1 H), 2.89–2.67 (m, 10 H), 2.35 (s, 3 H), 1.60 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$/CD_3OD, 2:1): δ = 166.0, 164.4, 151.3, 135.0, 133.8, 133.7, 129.6, 129.3, 129.2, 128.7, 128.6, 128.5, 111.5, 86.8, 85.5, 79.0, 77.1, 64.4, 60.5, 55.0, 54.0, 45.0, 11.9 ppm. MALDI-HRMS: *m*/*z* 601.2280 ([M + Na]$^+$, $C_{30}H_{34}N_4O_8Na^+$ calcd. 601.2269).

1-[2-*O*-*tert*-Butyldimethylsilyl-3,5-di-*O*-benzoyl-4-*C*-(*N*-methylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (59): A solution of nucleoside **58** (1.04 g, 1.80 mmol), DMAP (440 mg, 3.6 mmol), imidazole (0.73 g, 10.7 mmol) and *tert*-butyldimethylsilyl chloride (0.82 g, 5.4 mmol) in anhydrous DMF (18 cm^3) was stirred for 12 h at 36 °C. To the reaction mixture was added CH_2Cl_2 (100 cm^3) and washing was performed with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The organic phase was dried (Na_2SO_4), filtered, concentrated to dryness under reduced pressure and the residue was purified by column chromatography [6% (v/v) MeOH in CH_2Cl_2] to give the nucleoside **59** (1.06 g, 86%) as a white solid material. R_f 0.56 (MeOH/CH_2Cl_2, 15:85, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.92–7.89 (m, 2 H), 7.82–7.79 (m, 2 H), 7.48 (m, 1 H), 7.41–7.33 (m, 3 H), 7.26–7.21 (m, 2 H), 7.16 (br. s, 1 H, 6-H), 6.01 (d, *J* = 7.1 Hz, 1 H, 1′-H), 5.76 (d, *J* = 6.9 Hz, 1 H, 3′-H), 4.51 (dd, *J* = 7.0 and 7.1 Hz, 1 H, 2′-H), 4.38 (d, *J* = 12.1 Hz, 1 H, 5′-Ha), 4.24 (d, *J* = 12.2 Hz, 1 H, 5′-Hb), 2.88–2.60 [m, 6 H, 5′′-H and CH_2(piperazinyl)], 2.42 [br. s, 4 H, CH_2(piperazinyl)], 2.18 (s, 3 H, NCH_3), 1.49 (s, 3 H, 5-CH_3), 0.68 [s, 9 H, C(CH_3)$_3$], –0.15 (s, 6 H, 2 × SiCH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 165.9 and 165.8 (2 × *C*OC$_6$H$_5$), 163.6 (C-4), 150.6 (C-2), 134.7, 133.8, 133.7, 129.7, 129.4, 128.9, 128.8, 128.7, 111.8 (C-5), 85.3 (C-1′), 84.8 (C-4′), 79.2 (C-3′), 77.9 (C-2′), 64.8 (C-5′), 60.8 (C-5′′), 55.5 and 55.1 [CH_2(piperazinyl)], 45.9 (NCH_3), 25.5 [C(*C*H$_3$)$_3$], 17.9 [Si*C*(CH$_3$)$_3$], 12.0 (5-CH_3), –4.6 and –4.9 (2 × SiCH_3) ppm. MALDI-HRMS: *m*/*z* 715.3131 ([M + Na]$^+$, $C_{36}H_{48}N_4O_8SiNa^+$ calcd. 715.3134).

1-[2-*O*-*tert*-Butyldimethylsilyl-4-*C*-(*N*-methylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (60): A solution of nucleoside **59** (1.03 g 1.51 mmol) in saturated methanolic ammonia (50 cm^3) was stirred for 24 h at room temp. The solution was evaporated to dryness under reduced pressure. The residue was coevoporated with toluene (2 × 10 cm^3) and purified by column chromatography [10–12% (v/v) MeOH in CH_2Cl_2] to give nucleoside **60** (610 mg, 83%) as a white solid material. R_f 0.36 (MeOH/CH_2Cl_2, 15:85, v/v). ^{1}H NMR ($CDCl_3$): δ = 7.21 (s, 1 H, 6-H), 5.41 (d, *J* = 5.6 Hz, 1 H, 1′-H), 4.44 (dd, *J* = 5.4 and 5.4 Hz, 1 H, 2′-H), 4.02 (d, *J* = 5.2 Hz, 1 H, 3′-H), 3.74 (s, 2 H, 5′-H), 2.60–2.53 [m, 6 H, 5′′-H and CH_2(piperazinyl)], 2.39 [br. s, 4 H, CH_2(piperazinyl)], 2.21 (s, 3 H, NCH_3), 1.86 (s, 3 H, 5-CH_3), 0.80 (s, 9 H, C(CH_3)$_3$), 0.16 and –0.06 (2s, 3 H each, 2 × SiCH_3) ppm. ^{13}C NMR ($CDCl_3$): δ =163.8 (C-4), 150.7 (C-2), 138.5 (C-6), 111.4 (C-5), 92.4 (C-1′), 87.5 (C-4′), 80.6 and 80.2 (C-2′ and C-3′), 65.6 (C-5′), 62.3 (C-5′′), 55.3 and 55.0 [CH_2(piperazinyl)], 45.9 (NCH_3), 25.7 [C(*C*H$_3$)$_3$], 18.0 [Si*C*(CH$_3$)$_3$], 12.5 (5-CH_3), –4.5 and –4.9 (2 × SiCH_3) ppm. MALDI-HRMS: *m*/*z* 507.2608 ([M + Na]$^+$, $C_{22}H_{40}N_4O_6SiNa^+$ calcd. 507.2609).

1-[2-*O*-*tert*-Butyldimethylsilyl-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(*N*-methylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (61): Dimethoxytrityl chloride (485 mg, 1.43 mmol) was added in one portion

to a stirred solution of the nucleoside **60** (578 mg, 1.19 mmol) in anhydrous pyridine (10 cm^3). The reaction mixture was stirred for 12 h at room temp., methanol (0.2 cm^3) was added and stirring was continued for another 15 min. The mixture was concentrated to dryness under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 10 cm^3). The separated organic phase was dried (Na_2SO_4), filtered, concentrated to dryness under reduced pressure and coevaporated with toluene (2 × 2 cm^3). The crude product obtained was purified by column chromatography [2% MeOH in CH_2Cl_2, containing 0.5% Et_3N (v/v/v)] to afford nucleoside **61** as a white solid material (826 mg, 88%). R_f 0.47 (MeOH/CH_2Cl_2, 15:85, v/v). ^1H NMR ($CDCl_3$): δ = 7.41 (s, 1 H, 6-H), 7.32 (d, J = 7.0 Hz, 2 H), 7.21–7.13 (m, 7 H), 6.72 (d, J = 8.8 Hz, 4 H), 5.88 (d, J = 6.5 Hz, 1 H, 1′-H), 4.24 (dd, J = 6.2 and 6.3 Hz, 1 H, 2′-H), 4.00 (dd, J = 6.4 and 7.5 Hz, 1 H, 3′-H), 3.70 (s, 6 H, 2 × OCH_3), 3.27 (d, J = 10.8 Hz, 1 H, 5′-Ha), 3.23 (d, J = 10.7 Hz, 1 H, 5′-Hb), 2.74 (d, J = 8.5 Hz, 1 H, 3′-OH), 2.43–2.26 [m, 10 H, 5′′-H and CH_2(piperazinyl)], 2.13 (s, 3 H, NCH_3), 1.30 (s, 3 H, 5-CH_3), 0.77 [s, 9 H, $C(CH_3)_3$], 0.10 and –0.07 (2s, 3 H each, 2 × $SiCH_3$) ppm. ^{13}C NMR ($CDCl_3$): δ = 163.8, 159.0, 150.6, 144.1, 136.2, 135.1, 135.0, 130.2, 130.1, 128.2, 128.1, 127.4, 113.5, 111.3, 88.0, 87.1, 86.0, 81.0, 80.2, 64.7, 61.5, 55.3, 54.9, 45.9, 25.7, 18.0, 11.8, –4.4, –4.8 ppm. MALDI-HRMS: m/z 809.3901 ([M + Na]$^+$, $C_{43}H_{58}N_4O_8SiNa^+$ calcd. 809.3916).

1-{2-*O*-*tert*-Butyldimethylsilyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphanyl]-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(*N*-methylpiperazinyl)methyl-β-D-xylofuranosyl}thymine (62): 2-Cyanoethyl *N*,*N*-diisopropylphosphoramido chloridite (125 mg, 0.53 mmol) was added dropwise to a stirred solution of the nucleoside **61** (350 mg, 0.44 mmol) in a mixture of diisopropylethylamine (0.5 cm^3) and anhydrous CH_2Cl_2 (10 cm^3) at room temp. After stirring the mixture was for 6 h, the reaction mixture was diluted with CH_2Cl_2 (20 cm^3). Washing was performed with saturated aq. $NaHCO_3$ (2 × 10 cm^3). 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 [70–90% EtOAc in *n*-hexane, containing 0.5% Et_3N (v/v/v)] to give amidite **62** as a white solid material (400 mg, 91%). R_f 0.36 (EtOAc/light petroleum, 75:25, v/v). ^{31}P NMR ($CDCl_3$): δ = 151.6, 150.0 ppm.

1-[4-*C*-(*N*-Methylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (63): A solution of nucleoside **57a** (124 mg, 0.2 mmol) in saturated methanolic ammonia (10 cm^3) was stirred for 36 h at room temp. The solution was evaporated to dryness under reduced pressure, and the residue dissolved in distilled water (10 cm^3) and washing was performed with diethyl ether (3 × 10 cm^3). The separated aqueous phase was concentrated to dryness to afford nucleoside **64** (48 mg, 65%) as a white solid material. R_f 0.25 (MeOH/CH_2Cl_2, 15:85, v/v). ^1H NMR (CD_3OD): δ = 7.92 (s, 1 H, 6-H), 5.92 (d, J = 5.3 Hz, 1 H, 1′-H), 4.29–4.26 (m, 2 H, 2′-H and 3′-H), 3.75 (d, J = 11.9 Hz, 1 H, 5′-Ha), 3.61 (d, J = 11.1 Hz, 1 H, 5′-Hb), 2.66–2.53 [m, 9 H, 5′′-Ha and CH_2(piperazinyl)], 2.49 (d, J = 14.0 Hz, 1 H, 5′′-Hb), 2.32 (s, 3 H, NCH_3), 1.89 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR (CD_3OD): δ = 167.6, 153.0, 138.5, 111.7, 88.6, 87.8, 79.8, 78.8, 64.8, 62.0, 56.2, 55.4, 45.8, 12.5 ppm. MALDI-HRMS: m/z 393.1757 ([M + Na]$^+$, $C_{16}H_{26}N_4O_6Na^+$ calcd. 393.1745).

1-(4-*C*-Piperazinylmethyl-β-D-xylofuranosyl)thymine (64): A solution of nucleoside **57b** (2.11g, 3.0 mmol) in saturated methanolic ammonia (100 cm^3) was stirred for 36 h at room temp. The solution was evaporated to dryness under reduced pressure and then partitioned between distilled water (50 cm^3) and diethyl ether (50 cm^3). The aqueous phase was washed with diethyl ether (2 × 50 cm^3) and concentrated to dryness to afford nucleoside **64** (769 mg, 71%) as a white solid material. R_f 0.16 (MeOH/CH_2Cl_2, 15:85, v/v). ^1H NMR (CD_3OD): δ = 7.92 (s, 1 H, 6-H), 5.93 (d, J = 5.1 Hz, 1 H, 1′-H), 4.29–4.26 (m, 2 H, 2′-H and 3′-H), 3.75 (d, J = 11.8 Hz, 1 H, 5′-Ha), 3.62 (d, J = 12.0 Hz, 1 H, 5′-Hb), 2.93–2.89 [m, 4 H, CH_2(piperazinyl)], 2.72–2.62 [m, 4 H, CH_2(piperazinyl)], 2.55 (d, J = 14.1 Hz, 1 H, 5′′-Ha), 2.48 (d, J = 14.4 Hz, 1 H, 5′′-Hb), 1.89 (s, 3 H) ppm. ^{13}C NMR (CD_3OD): δ = 166.4, 153.0, 138.5, 111.7, 88.7, 87.9, 79.9, 78.9, 64.8, 62.8, 56.3, 46.5, 12.5 ppm. MALDI-HRMS: m/z 379.1582 ([M + Na]$^+$, $C_{15}H_{24}N_4O_6Na^+$ calcd. 379.1588).

1-[4-*C*-(*N*-Trifluoroacetylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (65): To a suspension of nucleoside **64** (713 mg, 2.0 mmol) in anhydrous methanol (25 cm^3) was added DMAP (122 mg, 1.0 mmol) and ethyl trifluoroacetate (568 mg, 4.0 mmol). The reaction mixture was stirred for 12 h at room temp. and then concentrated to dryness under reduced pressure. The residue obtained was purified by column chromatography [6–8% (v/v) MeOH in CH_2Cl_2] to afford nucleoside **65** as a white solid material (760 mg, 84%). R_f 0.21 (MeOH/CH_2Cl_2, 10:90, v/v); ^1H NMR ($CDCl_3$): δ = 7.59 (s, 1 H), 5.67 (d, J = 5.7 Hz, 1 H), 4.24 (dd, J = 5.9 and 6.1 Hz, 1 H), 4.12 (d, J = 6.3 Hz, 1 H), 3.63–3.47 (m, 6 H), 2.54–2.44 (m, 6 H), 1.71 (s, 3 H) ppm. ^{13}C NMR ($CDCl_3$): δ = 164.8, 155.6 (q, J = 35.5 Hz), 151.6, 137.3, 116.5 (q, J 287.8), 111.1, 88.2, 87.9, 79.5, 78.6, 64.4, 61.4, 54.8, 54.3, 45.9, 43.5, 12.4. MALDI-HRMS: m/z 475.1402 ([M + Na]$^+$, $C_{17}H_{23}F_3N_4O_7Na^+$ calcd. 475.1411).

1-[5-*O*-4,4′-Dimethoxytrityl-4-*C*-(*N*-trifluoroacetylpiperazinyl)-methyl-β-D-xylofuranosyl]thymine (66): To a solution of nucleoside **65** (724 mg, 1.6 mmol) in anhydrous pyridine (3 cm^3) was added dimethoxytrityl chloride (592 mg, 1.75 mmol) and the resulting mixture was were stirred for 12 h at room temp. MeOH (0.1 cm^3) was added and stirring was continued for 20 min. The mixture was concentrated to dryness under reduced pressure and the residue was dissolved in CH_2Cl_2 (25 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 25 cm^3). The separated organic phase was dried (Na_2SO_4), filtered, concentrated to dryness under reduced pressure and coevaporated with toluene (2 × 1.0 cm^3). The crude product obtained was purified by column chromatography [3–5% MeOH in CH_2Cl_2, containing 0.5% Et_3N (v/v/v)] to afford nucleoside **66** as a white solid material (1.07 g, 89%). R_f 0.33 (MeOH/CH_2Cl_2, 15:85, v/v). ^1H NMR ($CDCl_3$): δ = 7.38 (d, J = 7.6 Hz, 2 H), 7.36 (s, 1 H, 6-H), 7.28–7.15 (m, 7 H), 6.76 (d, J = 8.8 Hz, 4 H), 5.94 (d, J = 4.5 Hz, 1 H, 1′-H), 4.44 (dd, J = 4.1 and 4.3 Hz, 1 H, 2′-H), 4.22 (d, J = 4.4 Hz, 1 H, 3′-H), 3.69 (s, 6 H, 2 × OCH_3), 3.51–3.38 [m, 5 H, 5′-Ha and CH_2(piperazinyl)], 3.25 (d, J = 10.0 Hz, 1 H, 5′-Hb), 2.66–2.51 [m, 6 H, 5′′-H and CH_2(piperazinyl)], 1.30 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 164.3, 158.9, 158.8, 155.5 (q, J = 35.8 Hz), 151.8, 144.4, 137.0, 135.3, 135.2, 130.3, 130.2, 128.2, 128.1, 127.2, 127.1, 116.5 (q, J = 287.7 Hz), 113.4, 110.3, 89.5, 89.3, 87.5, 80.3, 78.5, 63.9, 60.7, 55.3, 54.6, 54.2, 46.0 (d, J = 20.3 Hz), 43.4, 12.1 ppm. MALDI-HRMS: m/z 777.2680 ([M + Na]$^+$, $C_{38}H_{41}F_3N_4O_9Na^+$ calcd. 777.2718).

1-[2-*O*-Acetyl-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(*N*-trifluoroacetylpiperazinyl)methyl-β-D-xylofuranosyl]thymine (67): Acetic anhydride (44 mg, 0.44 mmol) dissolved in CH_3CN (0.5 cm^3) was added dropwise to a stirred solution of nucleoside **66** (330 mg, 0.44 mmol) and DMAP (21 mg, 0.17 mmol) in CH_3CN (5 cm^3). The reaction mixture was stirred for 3 h at room temp. MeOH (0.2 cm^3) was added and stirring was continued for another 30 min. CH_2Cl_2 (25 cm^3) was added and the mixture was washed with saturated aq. $NaHCO_3$ (2 × 10 cm^3). The separated organic phase was dried (Na_2SO_4), filtered and concentrated to dryness under reduced pres-

sure. The crude product obtained was purified by column chromatography [60–66% EtOAc in light petroleum, containing 0.5% Et_3N (v/v/v)] to afford nucleoside **67** as a white solid material (252 mg, 72%). R_f 0.40 ($MeOH/CH_2Cl_2$, 10:90, v/v). ^1H NMR ($CDCl_3$): δ = 9.31 (br. s, 1 H, NH), 7.57 (d, J = 1.0 Hz, 1 H, 6-H), 7.46–7.43 (m, 2 H), 7.34–7.24 (m, 7 H), 6.85 (d, J = 8.6 Hz, 4 H), 6.16 (d, J = 7.4 Hz, 1 H, 1′-H), 5.73 (dd, J = 7.3 and 7.4 Hz, 1 H, 2′-H), 4.47 (d, J = 7.2 Hz, 1 H, 3′-H), 3.79 (s, 6 H, 2×OCH_3), 3.66–3.55 [m, 4 H, CH_2(piperazinyl)], 3.40 (d, J = 10.5 Hz, 1 H, 5′-Ha), 3.33 (d, J = 10.5 Hz, 1 H, 5′-Hb), 2.70–2.53 [m, 4 H, CH_2(piperazinyl)], 2.43 (s, 2 H, 5′′-H), 2.16 (s, 3 H, $COCH_3$), 1.37 (s, 3 H, 5-CH_3) ppm. ^{13}C NMR ($CDCl_3$): δ = 171.0, 163.8, 159.1, 159.0, 155.4 (d, J = 35.3 Hz), 151.1, 143.7, 135.9, 134.8, 134.7, 130.3, 130.2, 128.3, 128.2, 127.5, 116.5 (d, J = 287.9 Hz), 113.5, 112.1, 88.2, 86.7, 82.8, 78.9, 76.7, 64.2, 60.4, 55.4, 54.8, 54.4, 46.1, 43.6, 20.9, 11.7 ppm. MALDI-HRMS: m/z 819.2811 ([M + Na]$^+$, $C_{40}H_{43}F_3N_4O_{10}Na^+$ calcd. 819.2824).

1-{2-*O*-Acetyl-3-*O*-[2-cyanoethoxy(diisopropylamino)phosphanyl]-5-*O*-(4,4′-dimethoxytrityl)-4-*C*-(*N*-trifluoroacetylpiperazinyl)methyl-β-D-xylofuranosyl}thymine (68): 2-Cyanoethyl *N*,*N*-diisopropylphosphoramidochloridite (125 mg, 0.53 mmol) was added dropwise to a stirred solution of the nucleoside **67** (350 mg, 0.44 mmol) in a mixture of diisopropylethylamine (0.5 cm^3) and anhydrous CH_2Cl_2 (10 cm^3) at room temp. After stirring the resulting mixture was for 6 h, the reaction mixture was diluted with CH_2Cl_2 (20 cm^3). Washing was performed with saturated aq. $NaHCO_3$ (2×10 cm^3). 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 [70–90% EtOAc in *n*-hexane containing 0.5% Et_3N (v/v/v)] to give amidite **68** as a white solid material (400 mg, 91%). R_f 0.51, 0.56 ($MeOH/CH_2Cl_2$, 10:90, v/v); δ_p ($CDCl_3$) 154.6 and 152.9. MALDI-HRMS: m/z 1019.3859 ([M + Na]$^+$, $C_{49}H_{60}F_3N_6O_{11}PNa^+$ calcd. 1019.3902).

Synthesis, Deprotection and Purification of XNAs: All XNAs were prepared using the phosphoramidite approach on a Biosearch 8750 DNA synthesizer in 0.2 μmol scale on CPG solid supports (BioGenex). The stepwise coupling efficiencies for phosphoramidites **1**, **12**, **51a**, **51b**, **62** and **68** (10 min coupling time), the known α-L-LNA thymine amidite[9,13] and LNA adenine amidite[14] (used for the incorporation of monomers $^{\alpha L}\mathbf{T}^{\mathbf{L}}$ and $\mathbf{A}^{\mathbf{L}}$, respectively, 6 min coupling time) and of unmodified deoxynucleoside phosphoramidites (2 min coupling time) were >98% using 1*H*-tetrazole as activator (pyridine hydrochloride was used as activator when coupling phosphoramidites **1**, **12**, **51a**, **51b**, **62** and **68**). After standard deprotection and cleavage from the solid support using 32% aqueous ammonia (12 h, 55 °C), the XNAs were precipitated from ethanol. The composition of the XNAs was verified by MALDI-MS analysis and the purity (>80%) by capillary gel electrophoresis. MALDI-MS: m/z ([M – H]$^-$, found/calcd.): **ON8**, 10257/10257; **ON21**, 2765/2769; **ON22**, 2798/2801; **ON23**, 2797/2799; **ON24**, 2888/2891; **ON25**, 2880/2881; **ON26**, 3140/3137; **ON27**, 2866/2867; **ON28**, 3099/3095; **ON29**, 4211/4210; **ON30**, 4239/4242; **ON31**, 4795/4794; **ON32**, 4322/4324; **ON33**, 4710/4708; **ON34**, 4312/4310; **ON36**, 2723/2728; **ON37**, 2816/2820; **ON39**, 4362/4368; **ON40**, 3481/3483.

Thermal Denaturation Studies: The thermal denaturation experiments were performed on a Perkin–Elmer UV/Vis spectrometer fitted with a PTP-6 Peltier temperature-programming element using a medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) or a high salt buffer (10 mM sodium phosphate, 700 mM sodium chloride, 0.1 mM EDTA, pH 7.0) and concentrations of either 1.0 μM or 1.5 μM of the two complementary strands (assuming identical extinction coefficients of standard and modified nucleotides). During melting experiemnts, the absorbance was monitored at 260 nm while the temperature was raised at a rate of 1 °C per min. The melting temperatures (T_m values) of the complexes were determined as the maximum of the first derivatives of the melting curves obtained.

Supporting Information (see also footnote on the first page of this article): (ESI). Copies of ^{13}C NMR spectra of compounds **3** (β-anomer), **5a**, **9**, **10**, **15**, **16**, **20**–**23**, **25**, **27**–**29**, **32**, **33**, **36**–**39, 41**, **43**–**46, 47** (major isomer), **47** (minor isomer), **48a**, **48b**, **49a**, **49b**, **50a**, **50b**, **52**, **53a**, **53b**, **54a**, **55a**, **55b**, **56a**, **56b**, **57a**, **57b**, **58**–**61**, **66**, **67** and ^{31}P NMR spectra of compounds **7**, **12**, **31**, **51a**, **51b**, **62** and **68**.

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[36] <Crystal data for **54a**: $C_{14}H_{26}N_2O_5$, M_r = 302.37; crystal dimensions 0.23×0.20×0.15 mm; orthorhombic, space group $P2_12_12_1$, a = 8.3223(3), b = 10.4481(3), c = 17.7815(5) Å, V = 1546.14(8) Å^3; Z = 4; $F(000)$= 656, $\rho_{calcd.}$ = 1.299 Mg·m^{-3}; Bruker SMART APEX CCD diffractometer, Mo-K_α radiation (λ = 0.7107 Å); T = 296(2) K; theta range: 3.88 < θ < 28.29°; 11276 reflections measured, of which 2188 were independent and 2086 observed with $I > 2\sigma(I)$; μ(Mo-K_α) = 0.098 mm^{-1}. Refinement on F^2 using SHELXTL v.6.10; R_1 = 0.037 for $I > 2\sigma(I)$ and wR_2 = 0.097 (all data); goodness-of-fit, S = 1.05. H atoms bound to C were placed geometrically and refined using a riding model. The H atoms of the hydroxy groups were located in difference Fourier maps and refined freely with isotropic displacement parameters. The absolute structure could not be determined and Friedel pairs were merged prior to the final cycles of refinement. CCDC-255506 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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