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2′-Spiro ribo- and arabinonucleosides: synthesis, molecular modelling and incorporation into oligodeoxynucleotides †

B. Ravindra Babu,[a] Lise Keinicke,[b] Michael Petersen,[a] Claus Nielsen[c] and Jesper Wengel*[a]

[a] *Nucleic Acid Center, Department of Chemistry, University of Southern Denmark, DK-5230 Odense M, Denmark. E-mail: jwe@chem.sdu.dk; Fax: +45 66158780; Tel: +45 65502510*

[b] *Department of Chemistry, University of Copenhagen, Universitetsparken 5, Denmark, DK-2100 Copenhagen, Denmark*

[c] *Retrovirus Laboratory, Department of Virology, Statens Serum Institut, DK-2300 Copenhagen, Denmark*

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We have synthesized four conformationally restricted bicyclic 2′-spiro nucleosides *via* 2′-*C*-allyl nucleosides as key intermediates. The *ribo*-configured 2′-spironucleosides **9b** and **14b** were obtained by a convergent strategy starting from 2-ketofuranose **1** whereas the *arabino*-configured 2′-spironucleosides **21** and **27** were obtained by a linear strategy with a 2′-ketouridine derivative as starting material. The furanose ring of **9b/14b** adopts *N*-type conformations whereas the furanose ring of **21/27** exists as an $N \rightleftharpoons S$ equilibrium. These compounds showed no anti-HIV-1 activity or cytotoxicity. Incorporation of the four 2′-spironucleosides (as monomers **X4** and **X5**) into oligodeoxynucleotides was accomplished using the phosphoramidite approach on an automated DNA synthesizer. Irrespective of monomeric configuration, hybridization studies revealed that these 2′-spironucleotide monomers (**X4** and **X5**) induce decreased duplex thermostabilities compared with the corresponding DNA:DNA and DNA:RNA duplexes. Molecular modelling indicated that steric constraints are a possible reason for the lowered binding affinities of the modified oligodeoxynucleotides towards complementary single-stranded DNA and single-stranded RNA complements.

Introduction

Conformational restriction of the furanose ring of nucleosides, nucleotides and oligonucleotides has been intensively pursued in recent years, stimulated by the potential use of these molecules as therapeutic agents.[1–4] Especially revealing with respect to RNA-targeting has been the design and synthesis of oligonucleotide analogues containing nucleotide monomers with bicyclic furanose moieties.[4–9] Alternatively, conformational restriction of the furanose ring of nucleosides has been achieved by the introduction of alkyl groups at the 2′-*C*- or 2′-*O*-positions of the furanose ring leading to nucleosides[10–12] and oligonucleotides[13–15] of therapeutic interest. An as yet relatively unexplored strategy for conformational restriction is the synthesis of spironucleosides. A 2′-spirocyclopropane derivative of 2′-deoxyadenosine has been synthesized as a mechanistic probe for ribonucleotide reductases,[16] and 1′-spiro-[17], 3-spiro-[18] and 4′-spiro[19] ribonucleoside derivatives have been prepared as conformationally restricted derivatives. Herein we introduce 2′-spironucleoside derivatives (2′-*C*,2′-*O*-propano- and 2′-*C*,2′-*O*-ethano nucleosides) as novel conformationally restricted probes. The *ribo*-configured nucleosides **9b** and **14b** and the *arabino*-configured nucleosides **21** and **27** were prepared together with the corresponding phosphoramidite derivatives to be used for incorporation of monomers **X5-Ribo**, **X4-Ribo**, **X5-Ara** and **X4-Ara** into oligodeoxynucleotides. As a *C*-alkyl group prefers a pseudoequatorial orientation and an *O*-alkyl group an axial orientation, we anticipated preferred furanose ring comformations to be *N*-type (C3′-*endo*) for the *ribo*-configured nucleosides **9b** and **14b** and nucleotide monomers **X5-Ribo** and **X4-Ribo** and *S*-type (C2′-*endo*) for the *arabino*-configured nucleosides **21** and **27** and nucleotide monomers **X5-Ara** and **X4-Ara** (Fig. 1).

Results and discussion

Synthesis

Recent reports[20,21] describing the synthesis of 2′-*C*-alkyl ribonucleosides inspired us to choose a short, flexible and convergent approach towards the 2′-spiro ribonucleosides **9b** and **14b** (Scheme 1). The first step, addition of an allyl group to the β-face of the known 2-keto-tri-*O*-benzoyl-α-D-ribofuranose **1**[22] required the use of a chemoselective organometallic reagent able to add to ketone functionalities in the presence of esters. Organocerium compounds are mild reagents that are known to provide such selectivity.[23] We preferred the allyl group because it has the advantage of being a "hidden protecting group" as the double bond can be oxidatively cleaved or hydrated regioselectively and thus easily converted into a 2-hydroxyethyl or a 3-hydroxypropyl group. The reaction of allylMgBr–$CeCl_3$ with the 2-ketofuranose **1** resulted in chemo- and stereoselective addition of the allyl group. Due to *in situ* transesterification and anomerization, an anomeric mixture of tri-*O*-benzoyl-2′-*C*-allyl ribofuranoses was obtained that was benzoylated to afford the anomeric mixture of tetra-*O*-benzoyl ribofuranose **2**. At this stage, the configuration at C2 was tentatively assigned as shown in **2** based on the fact that the attack by the allyl group is more likely to occur from the less hindered β-face of the furanose ring. This hypothesis was later confirmed (see below). Furanose **2** was stereoselectively coupled with persilylated thymine using $SnCl_4$ as Lewis acid which also resulted in partial *O*2′-debenzoylation. Subsequent deprotection of this mixture with saturated methanolic ammonia provided the β-configured 2′-*C*-allyl ribonucleoside **3** in 29% overall yield from ketofuranose **1**. Selective O5′-dimethoxytritylation followed by O3′-silyl-

† Electronic supplementary information (ESI) available: Copies of ^{13}C NMR spectra of compounds **2**–**4**, **5a**, **5b**, **6a**, **6b**, **7a**, **8a**, **8b**, **9a**, **9b**, **11a**, **11b**, **12a**, **12b**, **13a**, **13b**, **14a**, **14b**, **16**, **19**, **22** and **24**, and ^{31}P NMR spectra of compounds **10**, **15**, **23** and **29**. See http://www.rsc.org/suppdata/ob/b3/b306354b/

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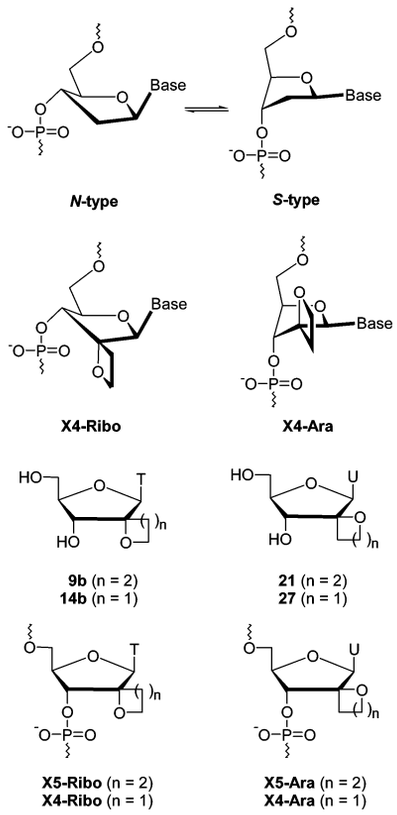
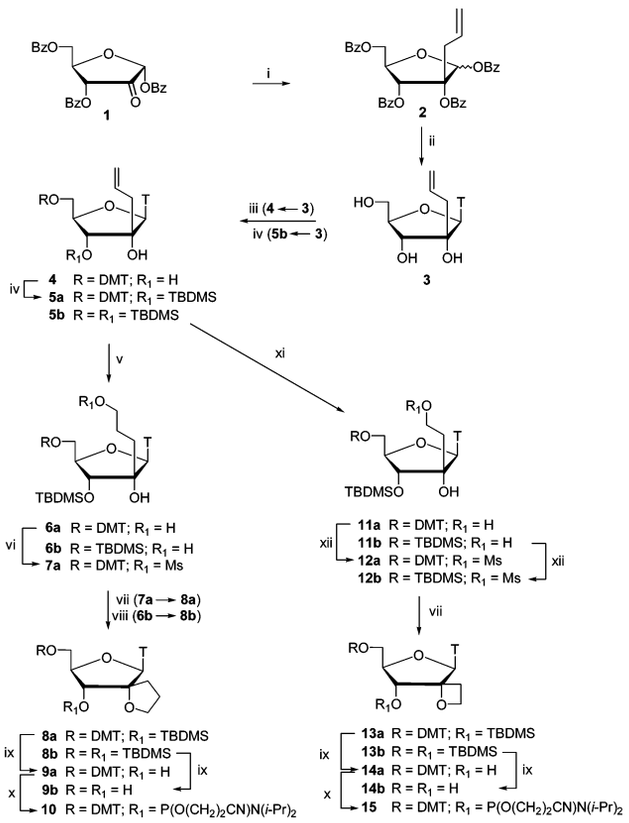


Fig. 1 Structures of the 2′-spironucleosides synthesized (**9b**, **14b**, **21**, **27**) and of the 2′-spironucleotide monomers incorporated into oligodeoxynucleotides (**X5** and **X4**). Also shown is the two-state conformational equilibrium of a 2′-deoxynucleoside and the anticipated *N*-type and *S*-type furanose conformations of the *ribo*- and *arabino*-configured 2′-spiro derivatives, respectively. For a detailed description of furanose conformations and the pseudorotational cycle the reader is referred to, *e.g.*, ref. 1.



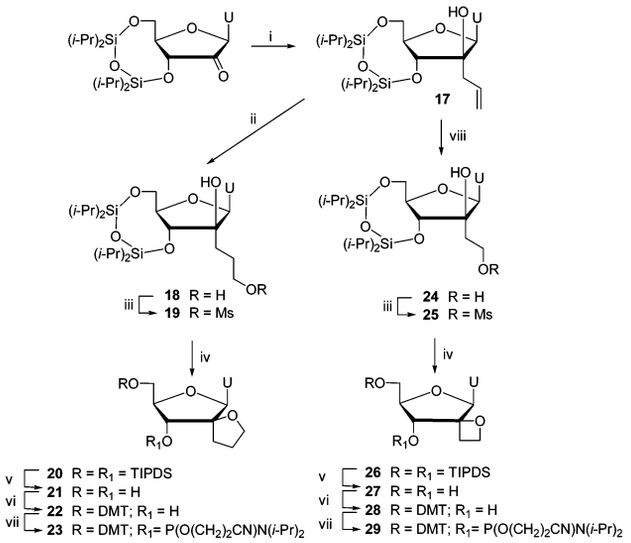
Scheme 1 *Reagents, conditions (and yields)*: i) a) allylMgBr, $CeCl_3$, THF, −78 °C, b) BzCl, DMAP, Et_3N, CH_2Cl_2 (53%); ii) a) thymine, $SnCl_4$, BSA, CH_3CN, b) saturated methanolic NH_3 (54%); iii) DMTCl, pyridine (86%); iv) TBDMSCl, imidazole, DMF (**5a**: 84%; **5b**: 84%); v) BH_3 (7.8 M in 1,4-oxathiane), THF, aq. NaOH (2 M), H_2O_2 (**6a**: 55%; **6b**: 39%); vi) MsCl, pyridine, CH_2Cl_2 (58%); vii) NaH, THF (**8a**: 87%; **13a**: 93%; **13b**: 77%); viii) a) MsCl, DMAP, Et_3N, CH_2Cl_2, b) NaH, THF (74%); ix) TBAF, THF (**9a**: 88%; **9b**: 78%; **14a**: 92%; **14b**: 79%); x) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2 (**10**: 76%; **15**: 80%); xi) a) OsO_4 (2.5 M in *n*-butanol), $NaIO_4$, THF, H_2O, b) $NaBH_4$, THF, H_2O (**11a**: 42%; **11b**: 47%). xii) MsCl, DMAP, Et_3N, CH_2Cl_2 (**12a**: 83%; **12b**: 64%). DMT = 4,4′-dimethoxytrityl. T = thymin-1-yl.

ation afforded the key intermediate **5a**. For the synthesis of the 2′-spiro ribonucleosides containing an additional O2′–C2′-linked five-membered ring, hydroboration of **5a** followed by alkaline hydrogen peroxide oxidation gave the anti-Markovnikov product **6a** exclusively in 55% yield. Attempts to cyclize this compound using Mitsunobu conditions failed. However, selective mesylation and subsequent treatment with sodium hydride induced cyclization to afford the 2′-spiro ribonucleoside **8a**. In order to obtain oligonucleotides (ONs) containing the nucleotide monomer X5-Ribo, O3′-desilylation to give **9a** followed by phosphitylation afforded the phosphoramidite building block **10** in 67% yield (Scheme 1).

For the synthesis of 2′-spiro ribonucleosides with an additional O2′–C2′-linked four-membered ring, the terminal double bond of compound **5a** was oxidatively cleaved using a catalytic amount of osmium tetraoxide and sodium periodate as a co-oxidant and then reduced by treatment with sodium borohydride resulting in the formation of the 2′-*C*-(2-hydroxy)-ethyl ribonucleoside **11a**. Selective mesylation followed by base-induced ring closure afforded in 77% yield the 2′-spiro ribonucleoside **13a**. Desilylation of compound **13a** followed by 3′-*O*-phosphitylation furnished the phosphoramidite building block **15** in 74% yield (Scheme 1).

Efforts to obtain the free dihydroxy nucleosides **9b** and **14b** for biological evaluation by detritylation of compounds **9a** and **14a** using either 10% dichloroacetic acid in dichloromethane or 90% acetic acid solution, in the presence of a DMT cation scavenger (triethylsilane), proved futile as analysis of the reaction mixture by analytical TLC showed the formation of several inseparable products. Therefore, in order to obtain **9b** and **14b**, compound **3** was O3′,O5′-disilylated to give nucleoside **5b**. Subsequent anti-Markovnikov hydration of the terminal double bond (to give **6b**), cyclization *via* the mesyl intermediate **7b** (to give **8b**) and, finally, desilylation afforded compound **9b** in 19% yield (from **3**). Similarly, oxidative cleavage of the terminal double bond of compound **5b**, followed by reduction, mesylation, intramolecular cyclization and desilylation afforded compound **14b** in 15% yield (from **3**). In addition, nucleoside **6b** was desilylated to provide 1-[2-*C*-(3-hydroxypropyl)-β-D-ribofuranosyl]thymine (**16**) in order to evaluate the effect of a 2′-*C*-hydroxypropyl group on biological activities.

The synthesis of the epimeric 2′-spiro arabinonucleosides **21** and **27** was accomplished using a linear strategy (Scheme 2). In general, addition reactions of alkyl groups to 2′-keto nucleosides using organometallic reagents usually proceed from the sterically less hindered α-face of the sugar ring.[24,25] Accordingly, Ce^{III}-assisted Grignard addition of the allyl group to 3′,5′-di-*O*-(tetraisopropylidinedisiloxane-1,3-diyl)-2′-ketouridine[26] occurred stereoselectively from the α-face of the furanose ring affording, after isolation and purification, diastereomerically pure key intermediate **17**. For the synthesis of 2′-spiro arabinonucleosides containing an additional O2′-C2′-linked five-membered ring, regioselective hydroboration of **17** was carried out using five equivalents of 9-BBN followed by oxidation with alkaline hydrogen peroxide (15 equivalents). Mesylation of the resulting 3′-*C*-(3-hydroxypropyl) arabinonucleoside **18** occurred selectively at the primary hydroxy group, and subsequent base-induced cyclization proceeded in 56% overall yield to give nucleoside **20** that was desilylated using two equivalents of $Et_3N{\cdot}3HF$ to give in 91% yield the 2′-spiro arabinonucleoside **21** ready for biological evaluation. O5′-Dimethoxytritylation (to give **22**) followed by O3′-phosphityl-



Scheme 2 *Reagents, conditions (and yields)*: i) allylMgBr, $CeCl_3$, THF, −78 °C (75%); ii) 9-BBN, THF, aq. NaOH (2 M), H_2O_2 (64%); iii) MsCl, DMAP, Et_3N, CH_2Cl_2 (**19**: 74%; **25**: 86%); iv) NaH, THF (**20**: 76%; **26**: 54%); v) $Et_3N{\cdot}3HF$, THF (**21**: 91%; **27**: 82%); vi) DMTCl, pyridine (**22**: 100%; **28**: 89%); vii) $NC(CH_2)_2OP(Cl)N(i\text{-}Pr)_2$, $EtN(i\text{-}Pr)_2$, CH_2Cl_2 (**23**: 61%; **29**: 60%); viii) a) OsO_4 (2.5 M in n-butanol), $NaIO_4$, THF, H_2O, b) $NaBH_4$, THF, H_2O (47%). DMT = 4,4′-dimethoxytrityl. U = uracil-1-yl.

ation using standard procedures afforded the phosphoramidite building block **23** (Scheme 2).

By oxidative cleavage followed by reduction, the allyl group of nucleoside **17** was converted into a 2-hydroxyethyl group furnishing the arabinonucleoside **24** in moderate yield. Subsequent selective mesylation, base induced intramolecular cyclization and desilylation (*via* **25** and **26**) afforded the 2′-spiro arabinonucleoside **27** containing an O2′–C2′-linked four-membered ring. Also this 2′-spironucleoside was prepared for incorporation into ODNs by standard dimethoxytritylation of the 5′-hydroxy group followed by phosphitylation of the 3′-hydroxy group to give the phosphoramidite building block **29** (*via* **28**) (Scheme 2).

Nucleoside configurations

The configurations of the 2′-spironucleosides were determined by NOE difference spectroscopy. Irradiation of H1′ of nucleoside **20** induced a 7% enhancement of H1″ (the protons of the 2′-*C*-methylene group) and *vice versa*, which suggests that these protons are positioned at the same side of the furanose ring. The *arabino*-configuration was further confirmed by the observation of strong NOE effects of H6 (6.5%) and H3″(8.5%) upon irradiation of H3′. Similarly in nucleoside **26**, mutual significant NOE contacts were observed between H1′ and H1″. Unfortunately, no mutual NOE contacts could be observed between the additional ring protons and the parent nucleoside in case of the 2′-spiro ribonucleoside series. The only key enhancement observed for these compounds was of H6 when irradiating H3′ (supporting *ribo*-configuration). Since these compounds were obtained following coupling between persilylated thymine and O2′-acylated ribofuranose **2**, the *trans* rule invoking anchimeric assistance from an O2′-acyl substituent, in this case supported by the exclusive isolation of one stereoisomer of nucleoside **3**, implies β-D-*ribo* configuration (*i.e.* *R*-configuration at C2 in compound **2**). The assigned configuration was furthermore confirmed by NOE experiments on the common intermediate **5b**. Significant NOE contacts were observed between H6, H3′ and H1″a (4.6%/4.9% between H6 and H3′, 1.1%/1.8% between H6 and H1″a, 1.8%/2.9% between H-3′ and H1″a) suggesting that these protons are positioned at the same side of the furanose ring. Furthermore, the NOE effects observed between the H6 and the H3′ protons (for **5b** as discussed above; **8a** (4.7%/5.9%); **13a** (5.3%/5.7%)) strongly support that both the monocyclic and the spirocyclic ribonucleosides adopt the expected *N*-type (C3′-*endo*) furanose conformation (see below).

Nucleoside conformations

Pseudorotation energy profiles were calculated by *ab initio* calculations at the MP2/cc-pVTZ level of theory for the **X4-Ribo** and **X4-Ara** nucleosides (compounds **14b** and **27**, respectively). For the nucleosides there are several possible conformations of the β, γ, and ε torsion angles.[27] In the calculations, these values were kept at values close to those expected upon incorporation into oligonucleotides and subsequent Watson–Crick hybridization.

X4-Ribo. Two local energy minima were found in the pseudorotation profile for this nucleoside (**14b**), these being $P = 13°$ and 159°, with the energy difference between these conformations depending on the value of the ε torsion angle (see Fig. 2). Using a generalized Karplus equation,[28–30] we found $J_{3'4'} = 8.5$ Hz and 0.8 Hz for these *N*-type and *S*-type sugar puckers, respectively. Experimentally $J_{3'4'} = 9.7$ Hz was found which shows that the **X4-Ribo** nucleoside is adopting an *N*-type furanose conformation in solution. Likewise, a large $J_{3'4'}$ was found experimentally for the **X5-Ribo** nucleoside (8.1 Hz). Although we have not carried out high-level calculations for this nucleoside, simple considerations show that this nucleoside adopts an *N*-type furanose conformation as well. Thus both the **X4-Ribo** and **X5-Ribo** nucleosides adopt *N*-type (RNA-like) furanose conformations.

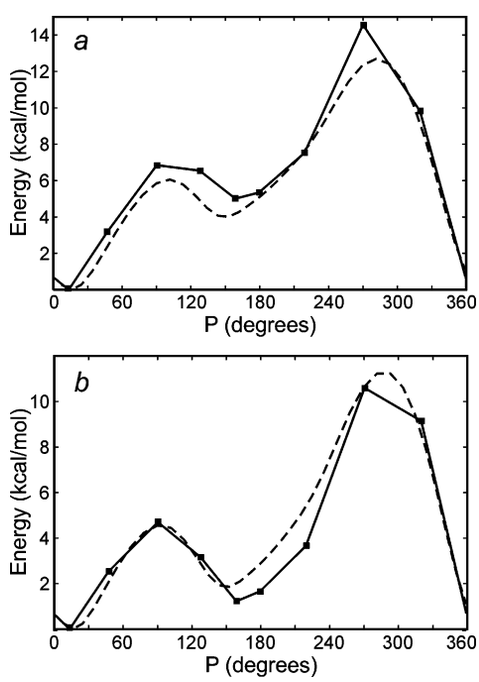


Fig. 2 MP2/cc-VTZ (full line) and force field (dotted line) potential energies (kcal mol^{-1}) as a function of pseudorotation angle *P* for **X4-Ribo**. All energies are given relative to the C3′-*endo* conformation. *a* With the ε torsion angle constrained to −124°. *b* With the ε torsion angle constrained to −165°.

X4-Ara. As for the corresponding *ribo*-configured compound, two energy minima for pseudorotation were found for this nucleoside (**27**) (P = 16° and 141°) (Fig. 2) with corresponding $J_{3'4'}$ coupling constants of 8.5 Hz and 1.8 Hz, respectively. The experimental value measured in solution was 6.1 Hz which shows that this nucleoside is not adopting a pure *N*- or *S*-type furanose conformation in solution but rather exists in an equilibrium between two (or more) conformations. Assuming an equilibrium between the two low energy conformations

Table 1 Thermal denaturation experiments (T_m values shown)[a]

Sequence (5′-3′)	DNA complement		RNA complement	
	Ribo series	Ara series	Ribo series	Ara series
ON1: T_7(**X5**)T_6	19	17	19	18
ON2: T_7(**X4**)T_6	20	18	19	19
ON3: T_5(**X5**)$_4T_5$	9	<5	8	<5
ON4: T_5(**X4**)$_4T_5$	8	<5	8	<5
ON5: T_{14}	32	32	29	29
ON6: (**X5**)$_9$T	<5	<5	<5	<5
ON7: (**X4**)$_9$T	<5	<5	<5	<5
ON8: T_{10}	20	20	18	18
ON 9: d[G(**X5**)GA(**X5**)A(**X5**)GC]	12	<5	n.m.	<5
ON10: d[G(**X4**)GA(**X4**)A(**X4**)GC]	12	<5	n.m.	<5
ON11: d(GTGATATGC)	29	29	n.m.	29

[a] Duplex melting temperatures (T_m values/°C) measured as the maximum of the first derivative of the melting curve (A_{260} *vs.* temperature) recorded in medium salt buffer (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0) using 1.5 μM concentrations of the two strands; A = adenin-9-yl monomer; C = cytosin-1-yl monomer; G = guanin-9-yl monomer; T = thymin-1-yl monomer; **X5** = 2′-*C*,2′-*O*-propano-linked 2′-spiro ribonucleotide monomer (**X5-Ribo**; indicated by "Ribo series" in the headline) or **X5** = 2′-*C*,2′-*O*-propano-linked 2′-spiro arabinonucleotide monomer (**X5-Ara**; indicated by "Ara series" in the headline); **X4** = 2′-*C*,2′-*O*-ethano-linked 2′-spiro ribonucleotide monomer (**X4-Ribo**; indicated by "Ribo series" in the headline) or **X4** = 2′-*C*,2′-*O*-ethano-linked 2′-spiro arabinonucleotide monomer (**X4-Ara**; indicated by "Ara series" in the headline). "n.m." = this experimental series not measured.

determined by calculations, the nucleoside possesses approximately 65% *N*-type conformation as gauged from the $J_{3'4'}$ coupling constant. This is in agreement with the quantum mechanical calculations, which predict the *N*-type pucker to be lower in energy than the *S*-type pucker, although a 65 : 35 distribution would give an energy difference of ~0.4 kcal mol^{-1}, which is far lower than the ~3.1 kcal mol^{-1} as determined by calculations. Assuming a single conformation of **X4-Ara** in solution, the measured $J_{3'4'}$ coupling constant would yield an O4′-*endo* ($P \simeq 90°$) furanose conformation. This conformation lies at the top of the pseudorotational energy barrier to interconversion and is, as such, unlikely. A rather similar $J_{3'4'}$ = 5.4 Hz was measured for **X5-Ara** in solution. This shows that the **X5-Ara** nucleoside adopts an equilibrium between two or more furanose conformations, possibly an $N \rightleftarrows S$ equilibrium as does **X4-Ara**.

Biological evaluation of the 2′-spironucleosides

The 2′-spironucleosides **9b**, **14b**, **21** and **27** and the 2′-alkyl-ribonucleosides **3** and **16** were evaluated for antiviral activity against HIV-1 in MT-4 cells (Medical Research Council, Centralised Facility for AIDS Reagents, UK) as described earlier.[31] All compounds were inactive against HIV-1 at 300 μM and displayed no cytotoxicity at 300 μM.

Syntheses of ONs and thermal denaturation studies

All oligomers **ON1**–**ON11** (Table 1) were prepared on a 0.2 μmol scale using the phosphoramidite approach. It should be noted that two variants of all modified ONs were prepared, *i.e.*, one in the "Ara series" and one in the "Ribo series" (see caption below Table 1). The stepwise coupling efficiencies of the phosphoramidites **10**, **15**, **23** and **29** (10 min coupling time) and of unmodified deoxynucleoside phosphoramidites were > 98% using 1*H*-tetrazole as activator (see experimental section for further details). After standard deprotection and ethanol precipitation, the composition of oligomers **ON1**–**ON4**, **ON6**, **ON7**, **ON9** and **ON10** was verified by MALDI-MS analysis and their purity (> 90%) by capillary gel electrophoresis.

Thermal denaturation studies towards complementary DNA and RNA target strands were performed in a medium salt buffer with 10-mer and 14-mer oligothymidylate sequences and with a mixed-base 9-mer sequence (Table 1). Regardless of sequence context and the number and structure of 2′-spironucleotides incorporated (**X5-Ribo**, **X4-Ribo**, **X5-Ara** and **X4-Ara**), significantly reduced thermal stabilities (duplex melting temperatures, T_m values) were obtained compared with the T_m values obtained for the unmodified reference duplexes (containing **ON5**, **ON8** and **ON11**). For the singly modified oligothymidylates **ON1** and **ON2**, the T_m values obtained were at least 10 °C lower than for the reference **ON5** with similar results obtained in the Ara and Ribo series. For the partly modified oligothymidylates **ON3** and **ON4**, and the partly modified mixed-base 9-mers **ON9** and **ON10**, T_m values corresponding to decreases of approximately 5 °C per 2′-spironucleotide monomer were obtained towards both the DNA and the RNA complements in the Ribo series. No melting transitions were observed for the corresponding Ara series of partly modified ONs which shows the inability of ONs containing a larger number of monomers **X5-Ara** and **X4-Ara** to form a duplex with natural ONs. The general and clear difference in melting behaviour between the partly modified ONs in the Ara and Ribo series cannot be explained by the presence of the thymine base in the Ribo series and the uracil base in the Ara series, but is more likely due to different conformational and steric effects (see next section). No melting transition was detected towards the DNA or RNA complements for the almost fully modified oligothymidylates **ON6** and **ON7**. In all melting experiments, similar results were obtained for the 2′-*C*,2′-*O*-ethano- and 2′-*C*,2′-*O*-propano-linked derivatives within an Ara or a Ribo series.

Modelling of X4-Ribo/DNA:DNA and X4-Ara/DNA:DNA duplexes

To assess the duplex geometry of **X4-Ribo** and **X4-Ara** modified dsDNA duplexes, we have carried out molecular dynamics (MD) simulations of the 5′-d(G**X**GA**X**A**X**GC)-3′:5′-d(GCATATCAC)-3′ duplex, with **X** being either **X4-Ribo-T** or **X4-Ara-U** modifications. The simulations were carried out using explicit solvent, periodic boundary conditions and Ewald treatment of electrostatic interactions. For each of the two duplexes, both standard A- and B-type duplex geometries were used as starting coordinates. This resulted in four simulations of at least 1 ns duration each. For comparison, two simulations (A- and B-form starting geometry; 1 ns) were carried out of the unmodified reference duplex. The calculations of the unmodified duplexes converged to identical structures (RMSD between the average structures from the final 500 ps of each simulation was 1.0 Å), and usual duplex features prevailed

throughout the simulation, *e.g.* normal Watson–Crick base pairing and a regular duplex geometry.

X4-Ribo/DNA:DNA. Two MD simulations were performed, starting from either A- or B-type duplex geometries. Both calculations produced stable trajectories as indicated by average RMSDs along the trajectories of 2.1 Å and 2.6 Å, respectively. The two calculations did not converge to identical structures for the **X4-Ribo** modified dsDNA duplex. In the B-structure, all sugars remain in *S*-type conformation, whereas in the A-structure, the three **X4-Ribo** furanoses remain in *N*-type conformations as does A4, while the deoxyriboses (except A4) repucker to *S*-type puckers (within ~400 ps). The difference in furanose conformation of the modified nucleotides is accompanied by differences in their ε torsion angles: in the A-type structure, we observe $\varepsilon = -124°$ whilst in the B-structure, the ε angles take values of $\sim -85°$ and $\sim -165°$. To investigate this phenomenon, we calculated pseudorotation energy profiles of the **X4-Ribo** nucleoside with two different values of the ε torsion angle ($-124°$ and $-165°$) at both force field and MP2/cc-pVTZ levels of theory (see details below and Fig. 2).

In both pseudorotation profiles, it is seen that the global energy minimum is *N*-type and that there is a local *S*-type minimum. Furthermore, the very close similarity between the energy profiles calculated at the two, very different, levels of theory (force field and *ab initio*) is striking, albeit fortuitous. The propensity of the modified sugars to remain in an *S*-type conformation in the B-structure is obvious from the pseudorotation profile calculated with $\varepsilon = -165°$ as there is a very deep local *S*-type minimum with an energy barrier to interconversion to *N*-type too large to be surmounted in the simulation time used (1 ns). A similar problem of incomplete conformational sampling is observed with stabilization of B-type RNA in MD simulations.[32] Therefore, we consider that the structure derived in the A-simulation is the structure most likely to be present in solution and thus we discuss the features of this structure. A view of the average structure from the final 1500 ps of the simulation is shown in Fig. 3. As can be observed, the presence of the C2′-spiro rings introduces pronounced kinks in the duplex owing to their steric clashes with primarily the O4′, O5′ and H5′ atoms of the 3′-flanking residue, with the bending at these base pair steps being approximately 30° towards the major groove. Consequently, the nucleobases of the modified nucleotides destack and tilt into the major groove and an irregular duplex structure ensues. Owing to the duplex bending into the major groove, the C2′-spiro rings are located at the brim of the minor groove, being exposed to the solvent there.

During the simulation, all deoxyriboses, with the exception of A4, repucker with populations of *S*-type conformation in the range 70 to 100%, except for C11 and C16, which have ~40% *S*-type conformation. The average minor groove width in the central part of the duplex is 9.5 Å, which is intermediate between A- and B-type duplex geometries as would be expected with the large proportion of *N*-type sugar conformations in the modified strand. The backbone angles of the duplex generally fall in the standard genus for right-handed nucleic acids, that is α to ζ: sc^-, ap, sc^+, sc^+ or ac^+, ap, sc^-, except the ε torsion angle in the modified nucleotides, which have values of $\sim -124°$. The global structure of the duplex is intermediate between A- and B-type geometries and highly irregular.

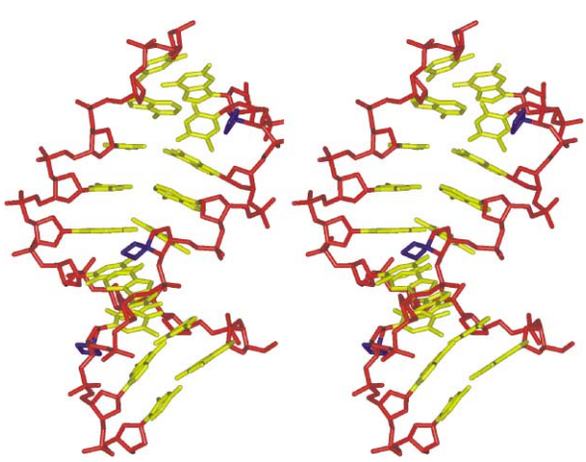


Fig. 3 MD simulation of **X4-Ribo**/DNA:DNA duplex. Stereo view of the average structure from the final 1500 ps of simulation. The colouring scheme used is: sugar–phosphate backbone, red; nucleobases, yellow; 2′-spiro-rings, blue.

Two of the modified **X4-Ribo** nucleotides partake in non-Watson–Crick hydrogen bonding at times in the simulation. After 1440 ps of simulation, the T7:A12 base pair is partly broken and T7 forms a bifurcated base pair with T13 and A12. Two hydrogen bonds are formed between T7 and T13 (T7:H3–T13:O4 and T7:O2–T13:H3) and the bond between A12:NH6 and T7:O4 remains intact (Fig. 4). For most of the duration of the bifurcated base pairing, A12 assumes an *N*-type sugar pucker. The bifurcated base pair persists for ~350 ps thereafter normal Watson–Crick base pairing resumes. While T7 and T13 base pair, A6 stacks with T7. In the T2:A17 base pair normal Watson–Crick base pairing is broken after 1065 ps and a non-Watson–Crick base pair is formed with hydrogen bonding between T2:O2 and A17:NH6. This hydrogen bond scheme remains for the duration of the simulation. In the remaining base pairs, usual Watson–Crick base pairing is observed.

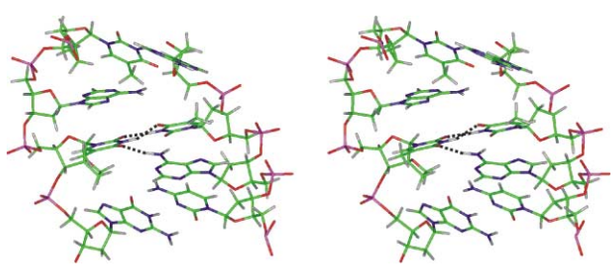


Fig. 4 MD simulation of **X4-Ribo**/DNA:DNA duplex. Stereo view of snapshot at 1600 ps showing the bifurcated base pair between T7 and A12/T13. Hydrogen bonds are shown as dotted lines. The colouring scheme used is: carbon, green; nitrogen, blue; oxygen, red; phosphorous, magenta; hydrogen, off-white.

As the control simulation of the unmodified dsDNA duplex showed no irregularities, *i.e.* pronounced bending of the duplex or non-Watson–Crick base pairing, these structural features are a consequence of the spiro modified nucleotides. Our MD simulation of the **X4-Ribo** modified dsDNA reveals convincingly that this modification fits poorly into the Watson–Crick framework of a nucleic acid duplex thereby also offering explanations for the negative influence of monomers **X4-Ribo** and **X5-Ribo** on duplex thermal stabilities.

X4-Ara/DNA:DNA. As the **X4-Ara** sugar is able to repucker, one must require the A- and B-simulations to converge in order to get a reliable picture of the geometry of the **X4-Ara** modified duplex. Unfortunately, our simulations did not converge, with the RMSD between the two average structures being 1.6 Å. This is probably a consequence of incomplete conformational sampling and longer trajectories would be required to alleviate this problem. Both simulations produce stable trajectories (RMSDs 2.2 Å and 2.6 Å for the A- and B-simulations, respectively). In the A-simulation, the deoxyriboses repucker to *S*-type conformations, as does one of the **X4-Ara** modified sugars, whilst in the B-simulation, all sugars, including the modified ones, remain in the *S*-type range. This suggests that in a duplex context, the *S*-type conformation is preferred by the modified sugars, although longer simulations would be required to establish this beyond doubt. In both simulations, normal Watson–Crick base pairing is observed throughout. As in the

X4-Ribo/DNA:DNA simulation, the spiro rings introduce some bending of the duplex at the base pair steps between the modified nucleotides and the 3′-flanking nucleotides, albeit the bending is less pronounced than in the **X4-Ribo** modified duplex. Altogether, it appears that the **X4-Ara** modifications introduce less distortion of the Watson–Crick duplex as compared with the **X4-Ribo** modification. However, to assess the structure in detail and its influence on thermal stabilities of duplexes, simulations longer than the present ones are required.

Conclusion

2′-Spiro nucleosides have been synthesized as a novel class of conformationally restricted nucleosides. The furanose ring of the *ribo*-configured 2′-spiro nucleosides adopts *N*-type conformations, whereas the furanose ring of the *arabino*-configured 2′-spiro nucleosides exists as an $N \rightleftharpoons S$ equilibrium. These 2′-spiro nucleosides and selected 2′-*C*-branched precursors did not show anti-HIV-1 activity or cytotoxicity. Irrespective of monomeric configuration, hybridization studies revealed that incorporation of these 2′-spironucleotide monomers induces decreased duplex thermostabilities compared to the corresponding unmodified reference duplexes. Molecular modelling clearly revealed unfavourable steric interactions as a plausible explanation for the lowered binding affinities.

Experimental

General

Reactions were conducted under an atmosphere of nitrogen when anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica plates with fluorescence indicator (SiO_2-60, F-254) visualizing under UV light and by spraying with 5% conc. 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. Petroleum ether of the distillation range 60–80 °C was used. After column chromatography fractions containing product were pooled, evaporated to dryness under reduced pressure and dried for 12 h under vacuum to give the product unless otherwise specified. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR spectra at 75.5 MHz, and ^{31}P NMR spectra at 121.5 MHz. Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as internal standard for ^{1}H NMR and ^{13}C NMR, and relative to 85% H_3PO_4 as external standard for ^{31}P NMR. Assignments of NMR spectra, when given, are based on 2D spectra and follow the standard carbohydrate/nucleoside nomenclature. Coupling constants (*J*-values) are given in Hertz. 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.

1,2,3,5-Tetra-*O*-benzoyl-2-*C*-allyl-D-ribofuranose (2)

$CeCl_3 \cdot 7H_2O$ (98.5%, 18.0 g, 48.3 mmol) was in a three-necked flask heated at 170 °C under vacuum for 4 h. The flask was purged with N_2 and after cooling on an ice bath, THF (140 cm^3, freshly distilled from sodium–benzophenone) was slowly added and stirring was continued for 12 h at rt. The suspension was cooled to −78 °C and allyl magnesium bromide (46.0 cm^3 of a 1.0 M solution in diethyl ether, 46 mmol) was added dropwise over 20 min and the resulting mixture stirred for another 2 h at −78 °C. A solution of 1,3,5-tri-*O*-benzoyl-2-keto-α-D-ribofuranose (**1**)[22] (4.80 g, 10.4 mmol) in THF (50 cm^3) was added over 15 min whereupon stirring was continued for 2 h at −78 °C. The ice–acetone cooling bath was removed and saturated NH_4Cl (100 cm^3) was added with stirring. After warming to rt, the mixture was filtered through Celite and washing was performed, successively, with diethyl ether (200 cm^3) and saturated aq. NH_4Cl (200 cm^3). The organic phase was separated and washed with saturated NH_4Cl (50 cm^3). The combined aq. phase was extracted with diethyl ether (100 cm^3), and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness to give a yellow oil. This oil was coevaporated with pyridine (2 × 5 cm^3) and the residue was dissolved in anhydrous CH_2Cl_2 (80 cm^3) whereupon DMAP (2.00 g, 16.4 mmol), benzoyl chloride (3.50 cm^3, 30.1 mmol) and Et_3N (100 cm^3) were added. After stirring for 12 h at rt, the reaction mixture was diluted with CH_2Cl_2 (100 cm^3) and then washed, successively, with 1 M aq. HCl (3 × 100 cm^3) and saturated aq. $NaHCO_3$ (50 cm^3). The combined aq. phase was extracted with diethyl ether (100 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure to give a yellow oil which was purified by column chromatography [90–95% (v/v) CH_2Cl_2 in petroleum ether] to yield furanose **2** (3.35 g, 53%, mixture of anomers) as a white foam; R_f 0.40, 0.35 (CH_2Cl_2); δ_H ($CDCl_3$, major anomer) 8.15–8.12 (2H, m), 8.07 (4H, d, *J* 7.8), 7.91–7.88 (2H, m), 7.66–7.59 (3H, m), 7.53–7.39 (7H, m), 7.19–7.14 (2H, m), 7.06 (1H, s), 6.12 (1H, d, *J* 8.0), 5.73 (1H, m), 5.09 (1H, dd, *J* 1.4 and 17.0), 4.90 (1H, dd, *J* 1.4 and 10.2), 4.79–4.68 (2H, m), 4.52 (1H, dd, *J* 4.5 and 12.0), 3.43 (1H, dd, *J* 7.3 and 14.9), 3.20 (1H, dd, *J* 7.6 and 14.9); δ_C ($CDCl_3$, major anomer) 166.2, 165.6, 164.8, 164.6, 133.8, 133.6, 133.0, 130.3, 130.2, 130.1, 130.0, 129.9, 129.7, 129.6, 129.5, 129.2, 129.1, 128.8, 128.7, 128.3, 120.0, 98.2, 88.5, 79.3, 75.7, 64.0, 35.3; MALDI-HRMS *m*/*z* 629.1755 ([M + Na]$^+$, $C_{36}H_{30}O_9Na^+$ calc. 629.1782).

1-(2-*C*-Allyl-β-D-ribofuranosyl)thymine (3)

N,*O*-Bis(trimethylsilyl)acetamide (25.0 cm^3, 101 mmol) was added to a suspension of furanose **2** (14.0 g, 23.1 mmol) and thymine (5.72 g, 45.4 mmol) in anhydrous acetonitrile (200 cm^3). The reaction mixture was refluxed for 1 h whereupon the clear solution was allowed to cool to rt. $SnCl_4$ (10 cm^3, 85.6 mmol) was added dropwise and the mixture was refluxed for 3 h. The mixture was then cooled to rt, EtOAc (250 cm^3) followed by saturated aq. $NaHCO_3$ (250 cm^3) were added and the mixture was stirred for 15 min. The mixture was filtered through Celite and washing was performed successively with EtOAc (200 cm^3) and saturated aq. $NaHCO_3$ (200 cm^3). The organic phase was separated and washed with saturated aq. $NaHCO_3$ (250 cm^3). The combined aq. phase was extracted with EtOAc (250 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The resulting yellow oil was purified by column chromatography [60–75% (v/v) EtOAc in petroleum ether] affording a white solid material. Saturated methanolic ammonia (150 cm^3) was added and stirring at rt was continued for 48 h. The mixture was evaporated under reduced pressure to give a residue that was coevaporated with acetonitrile (2 × 50 cm^3) and then purified by column chromatography [8–10% (v/v) MeOH in CH_2Cl_2] to give nucleoside **3** (3.71 g, 54%) as a white solid material; R_f 0.3 (CH_2Cl_2–MeOH 90 : 10, v/v); δ_H (CD_3OD) 7.85 (1H, s, H-6), 6.00 (1H, s, H-1′), 5.87 (1H, m, H-2″), 4.97 (1H, d, *J* 13.7, H-3″a), 4.96 (1H, d, *J* 13.1, H-3″b), 4.07 (1H, d, *J* 9.2, H-3′), 3.98 (1H, dd, *J* 1.9 and 12.7, H-5′a), 3.88 (1H, ddd, *J* 2.4, 2.4 and 9.2, H-4′), 3.79 (1H, dd, *J* 2.7 and 12.5, H-5′b), 2.39 (1H, dd, *J* 6.7 and 14.8, H-1″a), 2.22 (1H, dd, *J* 7.4 and 14.6, H-1″b), 1.87 (3H, s, 5-CH_3); δ_C (CD_3OD) 166.2, 152.6, 138.8 (C-6), 133.6 (C-2″), 117.9 (C-3″), 111.2, 92.4 (C-1′), 83.5 (C-4′), 81.4 (C-2′), 72.6 (C-3′), 60.5 (C-5′), 40.3 (C-1″), 12.4 (5-CH_3); MALDI-HRMS *m*/*z* 321.1049 ([M + Na]$^+$, $C_{13}H_{18}N_2O_6Na^+$ calc. 321.1057).

1-[2-*C*-Allyl-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]-thymine (4)

4,4′-Dimethoxytrityl chloride (3.42 g, 10.1 mmol) was added in one portion to a stirred solution of nucleoside **3** (2.5 g, 8.4 mmol) in anhydrous pyridine (20 cm^3). After stirring for 12 h at rt, methanol (0.2 cm^3) was added and the resulting mixture was evaporated to dryness under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The combined aq. phase was extracted with CH_2Cl_2 (50 cm^3). The combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography [4–5% MeOH in CH_2Cl_2, containing 0.5% Et_3N (v/v/v)] yielding nucleoside **4** as a white solid material (4.33 g, 86%); R_f 0.3 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 10.35 (1H, br s), 7.67 (1H, s), 7.43 (2H, d, *J* 7.7), 7.33–7.20 (7H, m), 6.83 (4H, d, *J* 8.8), 6.11 (1H, s), 5.91 (1H, m), 5.20 (1H, br s), 5.11 (1H, d, *J* 10.0), 5.08 (1H, d, *J* 18.3), 4.24 (1H, d, *J* 7.2), 4.06 (1H, d, *J* 8.1), 3.78 (6H, s), 3.59 (1H, d, *J* 10.5), 3.45 (1H, d, *J* 10.7), 3.10 (1H, d, *J* 7.3), 2.47 (1H, dd, *J* 6.8 and 14.4), 2.32 (1H, dd, *J* 7.3 and 14.1), 1.41(3H, s); δ_C ($CDCl_3$) 164.3, 158.8, 151.3, 144.5, 136.0, 135.7, 135.5, 131.7, 130.3, 128.4, 128.0, 127.2, 119.2, 113.3, 111.6, 92.5, 86.7, 82.2, 79.5, 72.8, 61.6, 55.3, 39.5, 11.8; MALDI-HRMS *m*/*z* 623.2347 ([M + Na]$^+$, $C_{34}H_{36}N_2O_8Na^+$ calc. 623.2364).

1-[2-*C*-Allyl-3-*O*-(*tert*-butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-β-D-ribofuranosyl]thymine (5a)

To a solution of nucleoside **4** (5.00 g, 8.30 mmol) in anhydrous DMF (25 cm^3) were added imidazole (2.80 g, 41.1 mmol) and *tert*-butyldimethylsilyl chloride (3.01 g, 20.0 mmol) and stirring was continued for 12 h at 36 °C. The reaction mixture was evaporated to dryness under reduced pressure and the residue obtained was dissolved in CH_2Cl_2 (100 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The combined aq. phase was extracted with CH_2Cl_2 (50 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [3–4% MeOH in CH_2Cl_2, containing 0.5% Et_3N (v/v/v)] affording nucleoside **5a** as a white solid material (5.01 g, 84%); R_f 0.39 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 8.98 (1H, br s), 7.51 (1H, s), 7.40 (2H, d, *J* 7.9), 7.32–7.22 (7H, m), 6.83 (4H, d, *J* 8.6), 6.11 (1H, s), 5.85 (1H, m), 5.06 (1H, d, *J* 10.4), 4.99 (1H, d, *J* 18.1), 4.31 (1H, d, *J* 7.7), 3.98 (1H, m), 3.79 (6H, s), 3.74 (1H, m), 3.28 (1H, dd, *J* 3.2 and 10.9), 3.23 (1H, s), 2.39 (1H, dd, *J* 6.5 and 14.8), 2.21 (1H, dd, *J* 7.1 and 14.8), 1.40 (3H, s), 0.79 (9H, s), 0.07 (3H, s), −0.27 (3H, s); δ_C ($CDCl_3$) 163.8, 158.9, 150.5, 144.1, 136.6, 135.3, 132.2, 130.4, 130.3, 128.6, 128.0, 127.3, 118.4, 113.3, 113.2, 113.1, 110.8, 91.7, 86.7, 81.7, 80.0, 73.6, 61.5, 55.4, 40.0, 25.8, 18.0, 11.6, −4.2; MALDI-HRMS *m*/*z* 737.3252 ([M + Na]$^+$, $C_{40}H_{50}N_2O_8SiNa^+$ calc. 737.3229).

1-[2-*C*-Allyl-3,5-di-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine (5b)

To a stirred solution of nucleoside **3** (800 mg, 2.68 mmol) in anhydrous DMF (8 cm^3) was added *tert*-butyldimethylsilyl chloride (1.62 g, 10.8 mmol) and imidazole (1.46 g, 21.4 mmol) and stirring was continued overnight at 36 °C. After the usual work-up procedure, the residue obtained was purified by column chromatography [40% (v/v) EtOAc in petroleum ether] affording nucleoside **5b** as a white solid material (1.19 g, 84%); R_f 0.28 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.02 (1H, br s, –NH), 7.19 (1H, s, H-6), 6.05 (1H, s, H-1″), 5.85 (1H, m, H-2″), 5.03 (1H, d, *J* 10.4, H-3″a), 4.96 (1H, d, *J* 17.4, H-3″b), 4.10 (1H, d, *J* 7.4, H-3′), 4.01 (1H, d, *J* 10.9, H-5′a), 3.84–3.80 (2H, m, H-4′ and H-5″b), 3.23 (1H, s, –OH), 2.29 (1H, dd, *J* 6.2 and 14.7, H-1″a), 2.11 (1H, dd, *J* 7.3 and 14.8, H-1″b), 1.93 (3H, s, 5-CH_3), 0.95 (9H, s, –$C(CH_3)_3$), 0.93 (9H, s, –$C(CH_3)_3$), 0.17 (3H, s, –$SiCH_3$), 0.16 (3H, s, –$SiCH_3$), 0.13 (6H, s, 2 × –$SiCH_3$); δ_C ($CDCl_3$) 163.7, 150.5, 136.3 (C-6), 132.2 (C-2″), 118.0 (C-3″), 110.4, 90.8 (C-1′), 82.3 (C-4′), 79.6 (C-2′), 73.0 (C-3′), 60.9 (C-5′), 39.6 (C-1″), 26.1 and 25.8 (2 × –$C(CH_3)_3$), 18.6 and 18.0 (2 × –$C(CH_3)_3$), 12.7 (5-CH_3), −4.1, −4.2, −5.1 and −5.2 (4 × –$SiCH_3$); MALDI-HRMS *m*/*z* 549.2791 ([M + Na]$^+$, $C_{25}H_{46}N_2O_6Si_2Na^+$ calc. 549.2787).

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrity)-2-*C*-(3-hydroxypropyl)]-β-D-ribofuranosyl]thymine (6a)

To a stirred solution of nucleoside **5a** (3.57 g, 5.0 mmol) in anhydrous THF (25 cm^3) was added dropwise BH_3 (1.0 cm^3 of a 7.8 M solution in 1,4-oxathiane, 7.8 mmol). The mixture was stirred for 12 h at rt whereupon it was cooled to 0 °C using an ice bath. A mixture of 2 M aq. NaOH (4.0 cm^3, 8.0 mmol) and aq. H_2O_2 (35%, 1.5 cm^3, 17.4 mmol) was added dropwise and the mixture was stirred for 2 h at rt. EtOAc (200 cm^3) was added and washing was performed, successively, with H_2O (2 × 50 cm^3) and saturated aq. $NaHCO_3$ (2 × 50 cm^3). The combined aq. phase was extracted with EtOAc (50 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [4% (v/v) MeOH in CH_2Cl_2] affording nucleoside **6a** as a white solid material (2.02 g, 55%); R_f 0.28 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.46 (1H, s), 7.54 (1H, s), 7.38 (2H, d, *J* 7.7), 7.33–7.23 (7H, m), 6.83 (4H, d, *J* 8.9), 6.19 (1H, s), 4.27 (1H, d, *J* 8.2), 3.99 (1H, m), 3.79 (6H, s), 3.75 (1H, m), 3.63 (1H, m), 3.53 (1H, m), 3.27 (1H, m), 3.24 (1H, s), 2.47 (1H, br s), 1.69–1.54 (4H, m), 1.33 (3H, s), 0.79 (9H, s), 0.10 (3H, s), −0.25 (3H, s); δ_C ($CDCl_3$) 164.1, 158.9, 150.9, 144.1, 136.4, 135.3, 130.5, 130.4, 128.6, 128.0, 127.4, 113.3, 113.2, 111.5, 91.0, 86.8, 81.4, 80.8, 74.3, 62.7, 61.5, 55.4, 31.4, 26.6, 25.8, 18.0, 11.6, −4.1, −4.2; MALDI-HRMS *m*/*z* 755.3349 ([M + Na]$^+$, $C_{40}H_{52}N_2O_9SiNa^+$ calc. 755.3334).

1-[3,5-Di-*O*-(*tert*-butyldimethylsilyl)-2-*C*-(3-hydroxypropyl)-β-D-ribofuranosyl]thymine (6b)

By the procedure described above for synthesis of nucleoside **6a**, reaction of nucleoside **5b** (600 mg, 1.14 mmol) with BH_3 (0.2 cm^3 of a 7.8 M solution in 1,4-oxathiane, 1.56 mmol) in anhydrous THF (8 cm^3) followed by oxidation with a mixture of 2 M aq. NaOH (0.8 cm^3, 1.6 mmol) and aq. H_2O_2 (35%, 0.3 cm^3, 3.5 mmol) afforded a residue that was purified by column chromatography [50–60% (v/v) EtOAc in petroleum ether] to yield nucleoside **6b** as a white solid material (242 mg, 39%); R_f 0.20 (CH_2Cl_2–MeOH 95 : 5, v/v);δ_H ($CDCl_3$) 9.22 (1H, br s), 7.23 (1H, d, *J* 1.1), 6.10 (1H, s), 4.07 (1H, d, *J* 7.3),.4.02 (1H, dd, *J* 1.5 and 11.7), 3.97–3.78 (2H, m), 3.61 (1H, m), 3.50 (1H, m), 3.27 (1H, s), 2.33 (1H, br s), 1.92 (3H, d, *J* 0.9), 1.68 (1H, m), 1.59–1.42 (3H, m), 0.94 (9H, s), 0.93 (9H, s), 0.17 (6H, s), 0.13 (6H, s); δ_C ($CDCl_3$) 163.9, 150.7, 136.3, 110.9, 90.4, 82.1, 80.4, 73.4, 62.6, 60.9, 30.1, 26.3, 26.2, 25.8, 18.7, 18.1, 12.8, −4.0, −4.1, −5.0, −5.2; MALDI-HRMS *m*/*z* 567.2879 ([M+Na]$^+$, $C_{25}H_{48}N_2O_7Si_2Na^+$ calc. 567.2892).

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-2-*C*-(3-methanesulfonyloxypropyl)-β-D-ribofuranosyl]thymine (7a)

A solution of methanesulfonyl chloride (115 mg, 1.0 mmol) in anhydrous CH_2Cl_2 (1.0 cm^3) was added dropwise at rt to a solution of nucleoside **6a** (600 mg, 0.82 mmol) in anhydrous pyridine (2.0 cm^3). After stirring for 12 h at rt, methanol (0.1 cm^3) was added and the resulting mixture was evaporated to dryness under reduced pressure and coevaporated with anhydrous toluene (2 × 5 cm^3). The residue was dissolved in CH_2Cl_2 (50 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 25 cm^3). The combined aq. phase was extracted with CH_2Cl_2 (50 cm^3). The combined organic phase was dried (Na_2SO_4),

filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [2.0% (v/v) MeOH in CH_2Cl_2] affording nucleoside **7a** as a white solid material (385 mg, 58%); R_f 0.33 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.22 (1H, s), 7.54 (1H, s), 7.38 (2H, d, *J* 8.2), 7.34–7.24 (7H, m), 6.84 (4H, d, *J* 9.0), 6.18 (1H, s), 4.27 (1H, d, *J* 8.0), 4.20 (2H, t, *J* 5.6), 3.97 (1H, d, *J* 7.8), 3.79 (6H, s), 3.76 (1H, m), 3.26 (1H, dd, *J* 3.1 and 11.0), 3.09 (1H, s), 2.96 (3H, s), 1.96 (1H, m), 1.84 (1H, m), 1.68 (1H, m), 1.50 (1H, m), 1.31 (3H, s), 0.80 (9H, s), 0.11 (3H, s), −0.25 (3H, s); δ_C ($CDCl_3$) 163.8, 159.0, 150.7, 143.9, 136.1, 135.2, 135.1, 130.6, 130.5, 130.4, 128.7, 128.0, 127.4, 113.3, 113.2, 111.9, 90.7, 86.9, 81.3, 80.6, 74.2, 70.1, 61.3, 55.4, 37.4, 30.9, 25.7, 23.3, 17.9, 11.4, −4.2; FAB-MS: *m*/*z* 810 [M^+].

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,2-*C*-propano-β-D-ribofuranosyl]thymine (8a)

A solution of nucleoside **7a** (120 mg, 0.15 mmol) in THF (1.0 cm^3) was added dropwise to a stirred suspension of NaH (60% in mineral oil, 20 mg, 0.5 mmol) in anhydrous THF (2.0 cm^3), at 0 °C. After stirring for 2 h at rt, ice-cold H_2O (10 cm^3) was slowly added followed by addition of CH_2Cl_2 (25 cm^3). The separated organic phase was washed with saturated aq. $NaHCO_3$ (2 × 20 cm^3). The combined aq. phase was extracted with CH_2Cl_2 (25 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [2.0–2.5% (v/v) MeOH in CH_2Cl_2] affording nucleoside **8a** as a white solid material (92 mg, 87%); R_f 0.37 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.10 (1H, s), 7.78 (1H, s), 7.38 (2H, d, *J* 6.8), 7.32–7.23 (7H, m), 6.83 (4H, d, *J* 8.9), 5.98 (1H, s), 4.21 (1H, d, *J* 9.3), 4.12–4.04 (2H, m), 3.91 (1H, dd, *J* 7.5 and 14.5), 3.82 (1H, m), 3.79 (6H, s), 3.28 (1H, dd, *J* 2.3 and 11.1), 2.10–1.76 (4H, m), 1.19 (3H, s), 0.75 (9H, s), 0.07 (3H, s), −0.27 (3H, s); δ_C ($CDCl_3$) 164.0, 158.9, 150.8, 144.0, 135.5, 135.3, 130.6, 130.5, 128.8, 128.0, 127.4, 113.2, 113.1, 113.0, 111.2, 91.2, 90.3, 86.9, 81.0, 73.1, 69.8, 61.2, 55.4, 29.4, 26.3, 25.7, 18.0, 11.4, −4.0, −4.2; MALDI-HRMS *m*/*z* 737.3213 ([M + Na]$^+$, $C_{40}H_{50}N_2O_8SiNa^+$ calc. 737.3229).

1-[3,5-Di-*O*-(*tert*-butyldimethylsilyl)-2-*O*,2-*C*-propano-β-D-ribofuranosyl]thymine (8b)

A solution of nucleoside **6b** (225 mg, 0.410 mmol) and DMAP (77 mg, 0.63 mmol) in Et_3N (1.0 cm^3) was stirred at rt for 15 min whereupon methanesulfonyl chloride (57 mg, 0.50 mmol) dissolved in CH_2Cl_2 (0.5 cm^3) was added dropwise. After stirring for 12 h at rt, methanol (0.1 cm^3) was added dropwise and the resulting mixture was evaporated to dryness under reduced pressure, and coevaporated with anhydrous toluene (2 × 2 cm^3). The residue was dissolved in CH_2Cl_2 (25 cm^3) and washed with saturated aq. $NaHCO_3$ (2 × 25 cm^3). The combined aq. phase was extracted with CH_2Cl_2 (25 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue was dissolved in THF (1.0 cm^3) and this solution was added dropwise to a stirred suspension of NaH (60% in mineral oil, 20 mg, 0.50 mmol) in anhydrous THF (2.0 cm^3) at 0 °C. After stirring for 2 h at rt, ice-cold H_2O (10 cm^3) was slowly added followed by addition of CH_2Cl_2 (25 cm^3). The separated organic phase was washed with saturated aq. $NaHCO_3$ (2 × 20 cm^3) and the combined aq. phase was extracted with CH_2Cl_2 (25 cm^3). The combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [40% (v/v) EtOAc in petroleum ether] affording nucleoside **8b** as a white solid material (161 mg, 74%); R_f 0.33 (CH_2Cl_2–MeOH 90 : 10, v/v); δ_H ($CDCl_3$) 9.33 (1H, br s), 7.43 (1H, d, *J* 1.0), 6.00 (1H, s), 4.11–3.99 (3H, m), 3.96–3.88 (2H, m), 3.82 (1H, dd, *J* 1.8 and 11.9), 1.95 (3H, s), 1.92–1.75 (3H, m), 1.63 (1H, m), 0.95 (9H, s), 0.92 (9H, s), 0.15 (3H, s), 0.14 (3H, s), 0.13 (3H, s), 0.12 (3H, s); δ_C ($CDCl_3$) 163.9, 150.6, 135.4, 110.9, 90.4, 90.1, 81.3, 72.2, 69.5, 60.4, 29.4, 26.3, 26.2, 25.8, 18.9, 18.1, 12.8, −4.0, −4.1, −4.7, −5.2; MALDI-HRMS *m*/*z* 549.2776 ([M + Na]$^+$, $C_{25}H_{46}N_2O_6Si_2Na^+$ calc. 549.2786).

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,2-*C*-propano-β-D-ribofuranosyl]thymine (9a)

Tetrabutylammonium fluoride (0.60 cm^3 of a 1.1 M solution in THF, 0.66 mmol) was added to a stirred solution of nucleoside **8a** (300 mg, 0.42 mmol) in anhydrous THF (2.0 cm^3). After stirring for 2 h at rt, anhydrous toluene (2.0 cm^3) was added and the mixture was evaporated under reduced pressure to one-fifth of its original volume. The residue obtained was purified by column chromatography [3.0% (v/v) MeOH in CH_2Cl_2] affording nucleoside **9a** as a white solid material (222 mg, 88%); R_f 0.27 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.16 (1H, s), 7.59 (1H, d, *J* 0.9), 7.43 (2H, d, *J* 8.5), 7.34–7.22 (7H, m), 6.83 (4H, d, *J* 9.2), 6.00 (1H, s), 4.24 (1H, dd, *J* 9.8 and 10.6), 4.08–4.01 (2H, m), 3.86 (1H, d, *J* 9.3), 3.78 (6H, s), 3.58 (1H, dd, *J* 1.5 and 10.7), 3.45 (1H, dd, *J* 3.3 and 11.1), 2.45 (1H, d, *J* 11.3), 2.04–1.94 (3H, m), 1.80 (1H, m), 1.36 (3H, s); δ_C ($CDCl_3$) 163.9, 158.8, 150.7, 144.5, 135.6, 135.5, 135.3, 130.3, 130.2, 128.4, 128.2, 128.0, 127.2, 113.3, 111.5, 90.2, 90.0, 86.8, 81.9, 72.3, 69.9, 61.3, 55.3, 29.6, 26.4, 11.7; MALDI-HRMS *m*/*z* 623.2348 ([M + Na]$^+$, $C_{34}H_{36}N_2O_8Na^+$ calc. 623.2364).

1-(2-*O*,2-*C*-Propano-β-D-ribofuranosyl)thymine (9b)

By the procedure described above for synthesis of nucleoside **9a**, desilylation of nucleoside **8b** (135 mg, 0.26 mmol) using tetrabutylammonium fluoride (0.77 cm^3 of a 1.0 M solution in THF, 0.77 mmol) in anhydrous THF (1.0 cm^3) furnished a residue that was purified by column chromatography [3.0% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **9b** as a white solid material (60 mg, 78%); R_f 0.21 (CH_2Cl_2–MeOH 90 : 10, v/v); δ_H ($(CD_3)_2SO$) 11.36 (1H, s, –NH–), 7.93 (1H, s, H-6), 5.75 (1H, s, H-1′), 5.25 (1H, t, *J* 4.8, 5′-OH), 5.01 (1H, d, *J* 8.1, H-3′), 3.88–3.69 (4H, m, H-4′, H-5′a, H-3″), 3.62 (1H, m, H-5′b), 1.87–1.79 (3H, m, H-1″a, H-2″), 1.75 (3H, s, 5-CH_3), 1.56 (1H, m, H-1″b); δ_C ($(CD_3)_2SO$) 163.6 (C-4), 150.7 (C-2), 135.9 (C-6), 109.1 (C-5), 89.9 (C-1′), 89.7 (C-2′), 81.9 (C-4′), 70.9 (C-3′), 68.9 (C-3″), 58.5 (C-5′), 28.7 (C-2″), 25.7 (C-1″), 12.3 (5-CH_3); MALDI-HRMS *m*/*z* 321.1063 ([M + Na]$^+$, $C_{13}H_{18}N_2O_6Na^+$ calc. 321.1057).

1-[3-*O*-(2-Cyanoethoxy)(diisopropylamino)phosphino-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,2-*C*-propano-β-D-ribofuranosyl]thymine (10)

2-Cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (119 mg, 0.50 mmol) was added dropwise to a stirred solution of the nucleoside **9a** (250 mg, 0.42 mmol) and *N*,*N*′-diisopropylethylamine (0.4 cm^3) in anhydrous CH_2Cl_2 (2.5 cm^3). After stirring for 12 h at rt, methanol (0.2 cm^3) was added and the mixture diluted with EtOAc (20 cm^3). Washing was performed with saturated aq. $NaHCO_3$ (2 × 10 cm^3) and the combined aq. phase was extracted with EtOAc (25 cm^3). The combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [60–75% EtOAc in *n*-hexane, containing 0.5% Et_3N (v/v/v)] yielding the phosphoramidite **10** as a white solid material (255 mg, 76%); R_f 0.29 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_P ($CDCl_3$) 152.6, 149.7.

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-2-*C*-(2-hydroxyethyl)-β-D-ribofuranosyl]thymine (11a)

To a stirred solution of nucleoside **5a** (1.43 g, 2.00 mmol) in a mixture of THF (10 cm^3) and H_2O (5 cm^3) was added sodium periodate (1.34 g, 6.26 mmol) followed by osmium tetraoxide

(0.27 cm^3 of a 2.5% solution in *tert*-butyl alcohol, 21.5 μmol) and stirring was continued for 2 h at rt. H_2O (25 cm^3) was added and extraction was performed with EtOAc (2 × 50 cm^3). The organic phase was washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3). The combined aq. phase was extracted with EtOAc (50 cm^3) and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was dissolved in a mixture of THF (10 cm^3) and H_2O (2 cm^3) and sodium borohydride (120 mg, 3.1 mmol) was added. The reaction mixture was stirred for 12 h at rt whereupon H_2O (25 cm^3) was added and extraction was performed with EtOAc (2 × 50 cm^3). The combined organic phase was washed with saturated aq. $NaHCO_3$ (2 × 50 cm^3), the combined aq. phase was extracted with EtOAc (50 cm^3), and the combined organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by column chromatography [4–5% (v/v) MeOH in CH_2Cl_2] affording nucleoside **11a** as a white solid material (608 mg, 42%); R_f 0.28 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.49 (1H, br s), 7.49 (1H, s), 7.38 (2H, d, *J* 6.6), 7.31–7.24 (7H, m), 6.83 (4H, d, *J* 8.6), 6.17 (1H, s), 4.30 (1H, d, *J* 8.0), 3.99 (1H, d, *J* 7.1), 3.79 (6H, s), 3.78–3.69 (3H, m), 3.46 (1H, s), 3.28 (1H, dd, *J* 3.1 and 11.0), 2.95 (1H, br s), 1.88 (1H, m), 1.73 (1H, m), 1.37 (3H, s), 0.80 (9H, s), 0.11 (3H, s), −0.23 (3H, s); δ_C ($CDCl_3$) 163.9, 158.9, 150.9, 144.1, 136.4, 135.3, 130.4, 130.3, 130.2, 128.6, 128.0, 127.3, 113.3, 113.2, 111.6, 91.3, 86.8, 81.4, 81.0, 74.3, 61.6, 58.3, 55.4, 36.2, 25.8, 18.0, 11.6, −4.2; MALDI-HRMS *m/z* 741.3160 ([M + Na]$^+$, $C_{39}H_{50}N_2O_9SiNa^+$ calc. 741.3178).

1-[3,5-Di-*O*-(*tert*-butyldimethylsilyl)-2-*C*-(2-hydroxyethyl)-β-D-ribofuranosyl]thymine (11b)

By the procedure described above for synthesis of nucleoside **11a**, oxidative cleavage of the terminal double bond in nucleoside **5b** (400 mg, 0.76 mmol) using osmium tetraoxide (0.11 cm^3 of a 2.5% solution in *tert*-butyl alcohol, 8.8 μmol) and sodium periodate (540 mg, 2.52 mmol) in a mixture of THF (4 cm^3) and H_2O (2 cm^3), followed by reduction with sodium borohydride (50 mg, 1.32 mmol), work-up and column chromatography [50–60% (v/v) EtOAc in petroleum ether] afforded nucleoside **11b** as a white solid material (190 mg, 47%); R_f 0.34 (CH_2Cl_2–MeOH 90 : 10, v/v); δ_H ($CDCl_3$) 9.44 (1H, br s), 7.19 (1H, d, *J* 1.0), 6.09 (1H, s), 4.12 (1H, d, *J* 6.8), 4.00 (1H, dd, *J* 1.2 and 11.4), 3.88–3.83 (2H, m), 3.79–3.77 (2H, m), 3.50 (1H, s), 3.01 (1H, br s), 1.91 (3H, s), 1.82 (1H, m), 1.65 (1H, m), 0.93 (18H, s), 0.18 (3H, s), 0.17 (3H, s), 0.12 (6H, s); δ_C ($CDCl_3$) 163.8, 150.8, 136.3, 111.0, 90.5, 82.5, 80.7, 73.4, 61.1, 58.3, 35.3, 26.1, 25.8, 18.7, 18.1, 12.8, −4.1, −4.2, −5.1, −5.3; MALDI-HRMS *m/z* 553.2731 ([M + Na]$^+$, $C_{24}H_{46}N_2O_7Si_2Na^+$ calc. 553.2736).

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-2-*C*-(2-methanesulfonyloxyethyl)-β-D-ribofuranosyl]thymine (12a)

By the procedure described above during synthesis of compound **8b**, mesylation of nucleoside **11a** (530 mg, 0.74 mmol) using a solution of methanesulfonyl chloride (95 mg, 0.83 mmol) in CH_2Cl_2 (0.5 cm^3) in the presence of DMAP (98 mg, 0.80 mmol) and Et_3N (2.0 cm^3) afforded a residue that was purified by column chromatography [3.0% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **12a** as a white solid material (491 mg, 83%); R_f 0.36 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.33 (1H, br s), 7.56 (1H, s), 7.38–7.23 (9H, m), 6.84 (4H, d, *J* 8.8), 6.12 (1H, s), 4.45–4.38 (2H, m), 4.33 (1H, d, *J* 8.2), 3.98 (1H, d, *J* 7.9), 3.79 (6H, s), 3.75 (1H, m), 3.29 (1H, dd, *J* 3.0 and 11.1), 3.15 (1H, s), 3.00 (3H, s), 2.10 (1H, m), 1.87 (1H, m), 1.28 (3H, s), 0.80 (9H, s), 0.12 (3H, s), −0.27 (3H, s); δ_C ($CDCl_3$) 163.8, 159.0, 150.9, 143.8, 135.1, 130.5, 130.4, 128.7, 128.0, 127.5, 113.3, 113.2, 112.2, 90.9, 87.0, 81.1, 79.8, 73.9, 66.5, 61.2, 55.4, 37.0, 34.1, 25.7, 17.9, 11.4, −4.3.

1-[3,5-Di-*O*-(*tert*-butyldimethylsilyl)-2-*C*-(2-methanesulfonyloxyethyl)-β-D-ribofuranosyl]thymine (12b)

By the procedure described above during synthesis of compound **8b**, mesylation of nucleoside **11b** (185 mg, 0.35 mmol) using a solution of methanesulfonyl chloride (57 mg, 0.50 mmol) in CH_2Cl_2 (0.5 cm^3) in the presence of DMAP (61 mg, 0.50 mmol) and Et_3N (1.0 cm^3) afforded a residue that was purified by column chromatography [40–50% (v/v) EtOAc in petroleum ether] to give nucleoside **12b** as a white solid material (135 mg, 64%); R_f 0.49 (CH_2Cl_2–MeOH 90 : 10, v/v); δ_H ($CDCl_3$) 9.25 (1H, s), 7.21 (1H, d, *J* 1.2), 6.06 (1H, s), 4.35 (2H, t, *J* 7.4), 4.12 (1H, d, *J* 7.2), 4.04 (1H, d, *J* 11.4), 3.86–3.79 (2H, m), 3.17 (1H, s), 2.99 (3H, s), 2.03 (1H, m), 1.94 (3H, s), 1.83 (1H, m), 0.94 (9H, s), 0.93 (9H, s), 0.19 (6H, s), 0.13 (6H, s); δ_C ($CDCl_3$) 163.6, 150.8, 135.9, 111.7, 90.3, 81.9, 79.4, 73.1, 66.1, 60.8, 37.0, 33.5, 26.2, 26.1, 25.8, 18.8, 18.0, 12.9, −4.1, −4.2, −4.9, −5.2; MALDI-HRMS *m/z* 631.2484 ([M + Na]$^+$, $C_{25}H_{48}N_2O_9SSi_2Na^+$ calc. 631.2511).

1-[3-*O*-(*tert*-Butyldimethylsilyl)-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,2-*C*-ethano-β-D-ribofuranosyl]thymine (13a)

By the procedure described above for synthesis of compound **8a**, cyclization of nucleoside **12a** (680 mg, 0.85 mmol) using NaH (60% suspension in mineral oil, 100 mg, 2.5 mmol) in anhydrous THF (2.0 cm^3) afforded a residue that was purified by column chromatography [2.0–3.0% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **13a** as a white solid material (556 mg, 93%); R_f 0.35 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.09 (1H, s, –NH–), 7.69 (1H, d, *J* 1.1, H-6), 7.39–7.23 (9H, m), 6.83 (4H, d, *J* 9.0), 6.29 (1H, s, H-1′), 4.66 (1H, dd, *J* 1.2 and 5.6, H-2″a), 4.46 (1H, dd, *J* 2.5 and 5.8, H-2″b), 4.22 (1H, d, *J* 9.2, H-3′), 3.91 (1H, d, *J* 9.0, H-4′), 3.79 (6H, s, 2 × –OCH_3), 3.77 (1H, m, H-5′a), 3.25 (1H, dd, *J* 2.6 and 11.0, H-5′b), 2.62–2.54 (2H, m, H-1″), 1.26 (3H, s, 5-CH_3), 0.82 (9H, s, –$C(CH_3)_3$), 0.17 (3H, s, –Si–CH_3), −0.20 (3H, s, –Si–CH_3); δ_C ($CDCl_3$) 163.9, 158.9, 150.8, 144.0, 135.7, 135.3, 135.2, 130.5, 130.4, 128.7, 128.0, 127.4, 113.3, 113.2, 111.3, 91.7 (C-1′), 91.4 (C-2′), 86.9 (Ar_3C), 79.7 (C-4′), 72.9 (C-3′), 66.9 (C-2″), 61.2 (C-5′), 55.3 (2 × –OCH_3), 25.7 (–$C(CH_3)_3$), 24.8 (C-3″), 18.1 (–$C(CH_3)_3$), 11.5 (5-CH_3), −4.0 (–Si–CH_3), −4.3 (–Si–CH_3); MALDI-HRMS *m/z* 723.3059 ([M + Na]$^+$, $C_{39}H_{48}N_2O_8SiNa^+$ calc. 723.3072).

1-[3,5-Di-*O*-(*tert*-butyldimethylsilyl)-2-*O*,2-*C*-ethano-β-D-ribofuranosyl]thymine (13b)

By the procedure described above for synthesis of compound **8a**, cyclization of nucleoside **12b** (115 mg, 0.19 mmol) using NaH (60% suspension in mineral oil, 40 mg, 1.0 mmol) in anhydrous THF (1.5 cm^3) afforded a residue that was purified by column chromatography [40% (v/v) EtOAc in petroleum ether] to yield nucleoside **13b** as a white solid material (74 mg, 77%); R_f 0.35 (CH_2Cl_2–MeOH 90 : 10, v/v); δ_H ($CDCl_3$) 9.24 (1H, s), 7.28 (1H, d, *J* 1.0), 6.24 (1H, s), 4.58 (1H, m), 4.47 (1H, m), 4.08–4.02 (2H, m), 3.82–3.73 (2H, m), 2.55 (1H, m), 2.45 (1H, m), 1.93 (3H, d, *J* 1.2), 0.98 (9H, s), 0.94 (9H, s), 0.23 (3H, s), 0.19 (3H, s), 0.12 (6H, s); δ_C ($CDCl_3$) 163.9, 150.6, 135.7, 111.0, 91.4, 90.8, 80.1, 72.4, 66.7, 60.5, 29.8, 26.2, 25.8, 25.2, 18.8, 18.2, 12.7, −4.1, −4.3, −4.9, −5.2; MALDI-HRMS *m/z* 535.2602 ([M + Na]$^+$, $C_{24}H_{44}N_2O_6Si_2Na^+$ calc. 535.2630).

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,2-*C*-ethano-β-D-ribofuranosyl]thymine (14a)

By the procedure described above for synthesis of compound **9a**, desilylation of nucleoside **13a** (550 mg, 0.78 mmol) with tetrabutylammonium fluoride (1.0 cm^3 of a 1.0 M solution in THF, 1.0 mmol) in THF (2.0 cm^3) afforded a residue that was purified by column chromatography [3.0–3.5% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **14a** as a white solid material (424 mg, 92%); R_f 0.25 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.35

(1H, s, –NH–), 7.51 (1H, s, H-6), 7.42 (2H, d, *J* 7.6), 7.32–7.16 (7H, m), 6.83 (4H, d, *J* 9.0), 6.29 (1H, s, H-1′), 4.76 (1H, dd, *J* 7.9 and 13.7, H-2″a), 4.50 (1H, dd, *J* 5.7 and 14.4, H-2″b), 4.25 (1H, dd, *J* 9.9 and 10.6, H-3′), 3.78 (6H, s, 2 × –OCH_3), 3.74 (1H, m, H-4′), 3.58 (1H, dd, *J* 1.6 and 10.9, H-5′a), 3.44 (1H, dd, *J* 2.8 and 11.0, H-5′b), 2.95 (1H, d, *J* 11.1, 3′-OH), 2.82 (1H, m, H-3″a), 2.51 (1H, m, H-3″b), 1.41 (3H, s, 5-CH_3); δ_C ($CDCl_3$) 163.9, 158.8, 150.7, 144.5, 135.6, 135.5, 130.3, 130.2, 128.3, 128.0, 127.2, 113.3, 111.5, 92.1 (C-2′), 90.5 (C-1′), 86.8 (Ar_3C–), 80.5 (C-4′), 72.3 (C-3′), 67.9 (C-2″), 61.3 (C-5′), 55.3 (2 × –OCH_3), 24.9 (C-3″), 11.8 (5-CH_3); MALDI-HRMS *m*/*z* 609.2198 ([M+Na]$^+$, $C_{33}H_{34}N_2O_8Na^+$ calc. 609.2207).

1-(2-*O*,2-*C*-Ethano-β-D-ribofuranosyl)thymine (14b)

By the procedure described above for synthesis of compound **9a**, desilylation of nucleoside **13b** (60 mg, 0.12 mmol) with tetrabutylammonium fluoride (0.15 cm^3 of a 1.0 M solution in THF, 0.15 mmol) in THF (1.0 cm^3) afforded a residue that was purified by column chromatography (EtOAc) to yield nucleoside **14b** as a white solid material (26 mg, 79%); R_f 0.25 (CH_2Cl_2–MeOH 90 : 10, v/v); δ_H (CD_3OD + $CDCl_3$, 3 : 1) 7.80 (1H, d, *J* 0.9), 6.22 (1H, s), 4.67–4.51 (2H, m), 4.06 (1H, d, *J* 9.7), 4.01 (1H, dd, *J* 2.1 and 12.6), 3.81 (1H, dd, *J* 2.5 and 12.6), 3.72 (1H, ddd, *J* 2.3, 2.3 and 9.5), 2.72 (1H, ddd, *J* 6.9, 8.6 and 11.8), 2.51 (1H, ddd, *J* 6.3, 8.7 and 11.7), 1.89 (3H, d, *J* 1.0); δ_C (CD_3OD + $CDCl_3$, 3 : 1) 165.4, 151.7, 137.3, 111.2, 92.6, 91.0, 81.3, 71.7, 67.6, 59.7, 25.0, 12.4; MALDI-HRMS *m*/*z* 307.0897 ([M + Na]$^+$, $C_{12}H_{16}N_2O_6Na^+$ calc. 307.0901).

1-[3-*O*-(2-Cyanoethoxy)(diisopropylamino)phosphino-5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,2-*C*-ethano-β-D-ribofuranosyl]thymine (15)

By the procedure described above for synthesis of compound **10**, treatment of compound **14a** (386 mg, 0.66 mmol) with 2-cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (166 mg, 0.70 mmol) in the presence of *N*,*N*′-diisopropylethylamine (0.4 cm^3) and anhydrous CH_2Cl_2 (3.0 cm^3) afforded a residue that was purified by column chromatography [60–75% EtOAc in *n*-hexane, containing 0.5% Et_3N (v/v/v)] to yield phosphoramidite **15** as a white solid material (412 mg, 80%). R_f 0.32 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_P ($CDCl_3$) 152.4, 149.6.

1-[2-*C*-(3-Hydroxypropyl)-β-D-ribofuranosyl]thymine (16)

By the procedure described above for synthesis of compound **9a**, desilylation of nucleoside **6b** (55 mg, 0.1 mmol) with tetrabutylammonium fluoride (0.20 cm^3 of a 1.0 M solution in THF, 0.20 mmol) in THF (1.0 cm^3) afforded a residue that was purified by column chromatography [15% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **16** as a white solid material (25 mg, 78%); R_f 0.23 (CH_2Cl_2/MeOH 85:15, v/v); δ_H (CD_3OD) 7.92 (1H, s), 6.04 (1H, s), 4.05 (1H, d, *J* 9.0), 4.00 (1H, d, *J* 12.2), 3.90 (1H, d, *J* 8.8), 3.81 (1H, d,*J* 12.3), 3.51–3.49 (2H, m), 3.37 (1H, s), 3.34 (1H, br s), 1.89 (3H, s), 1.83–1.52 (4H, m); δ_C (CD_3OD) 166.2, 152.6, 138.8, 111.4, 93.0, 84.0, 81.4, 73.4, 63.2, 60.5, 32.4, 26.9, 12.5.

1-[2-*C*-Allyl-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil (17)

$CeCl_3 \cdot 7H_2O$ (20.0 g, 53.7 mmol) was added to a three-necked flask and heated at 140 °C under vacuum for 12 h. The flask was purged with N_2 and after cooling on an ice bath, THF (150 cm^3, freshly distilled from sodium–benzophenone) was slowly added and stirring was continued for 2 h at rt. The suspension was cooled to −78 °C and allyl magnesium bromide (53.7 cm^3 of a 1.0 M solution in diethyl ether, 53.7 mmol) was added dropwise over 20 min and the mixture was stirred for an additional 2 h. A solution of 3′,5′-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2′-ketouridine[26] (5.95 g, 12.3 mmol) in anhydrous THF (20 cm^3) was added dropwise at −78 °C and the mixture was stirred for another 2 h. The cooling bath was removed and glacial acetic acid (10 cm^3) was poured into the reaction mixture. After being allowed to warm to rt, ethyl acetate (400 cm^3) was added and washing was performed, successively, with H_2O (2 × 200 cm^3) and brine (2 × 200 cm^3). The organic phase was dried (Na_2SO_4), filtered and evaporated to dryness under reduced pressure to give a residue that was purified by column chromatography [0–4% (v/v) CH_3OH in CH_2Cl_2) yielding nucleoside **17** as a white solid material (4.84 g, 75%); R_f 0.55 (CH_2Cl_2–MeOH 92 : 8, v/v); δ_H ($CDCl_3$) 9.47 (1H, br s, –NH–), 7.82 (1H, d, *J* 8.2, H-6), 6.08 (1H, m, H-2″), 5.91 (1H, s, H-1′), 5.69 (1H, dd, *J* 1.3 and 8.0, H-5), 5.36.5–27 (2H, m, H-3″), 4.24 (1H, d, *J* 9.3, H-3′), 4.14 (1H, dd, *J* 1.6 and 13.2, H-5′a), 3.99 (1H, dd, *J* 2.9 and 13.5, H-5′b), 3.79 (1H, ddd, *J* 1.9, 2.6 and 9.3, H-4′), 2.72 (1H, m, H-1″a), 2.51 (1H, m, H-1″b), 1.13–0.97 (28H, m, –$SiCH(CH_3)_2$); δ_C 163.5 (C-4), 151.1 (C-2), 140.4 (C-6), 131.1 (C-2″), 121.8 (C-3″), 101.4 (C-5), 87.4 (C-1′), 80.8 (C-4′), 78.8 (C-2′), 73.0 (C-3′), 60.2 (C-5′), 37.8 (C-1″), 17.4, 17.3, 17.2, 17.1, 17.0 and 16.7 ($SiCH(CH_3)_2$), 13.4, 12.9, 12.8, and 12.3 ($SiCH(CH_3)_2$); FAB-MS *m*/*z* 527 [M + H]$^+$.

1-[2-*C*-(3-Hydroxypropyl)-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil (18)

To a stirred solution of nucleoside **17** (2.21 g, 4.20 mmol) in anhydrous THF (15 cm^3) was added 9-borabicyclo[3.3.1]-nonane (42.0 cm^3 of a 0.5 M solution in THF, 21.0 mmol). The mixture was stirred for 3 h at rt, whereupon it was cooled to 0 °C and 2 M aq. NaOH (10.5 cm^3, 21.0 mmol) and 35% aq. H_2O_2 (6.30 cm^3, 63.0 mmol) were added slowly. The mixture was allowed to warm to rt, stirred for 2 h and then poured into a mixture of diethyl ether (150 cm^3) and H_2O (150 cm^3). The aq. phase was extracted with diethyl ether (50 cm^3), the organic extracts were combined with the separated organic phase and washed, successively, with saturated aq. $NaHCO_3$ (2 × 40 cm^3) and H_2O (2 × 40 cm^3), dried ($MgSO_4$), filtered and evaporated to dryness under reduced pressure. The residue obtained was purified by dry column chromatography[33] [0–3% (v/v) MeOH in CH_2Cl_2] yielding nucleoside **18** as a white solid material which was used in the next step without further purification (1.45 g, 64%); R_f 0.38 (CH_2Cl_2–MeOH 92 : 8, v/v); δ_H ($CDCl_3$) 10.76 (1H, br s, NH), 7.99 (1H, d, *J* 8.2, H-6), 5.85 (1H, s, H-1′), 5.70 (1H, dd, *J* 1.8 and 8.1, H-5), 4.16–4.12 (2H, m, H-3′ and H-5′a), 3.99–3.94 (2H, m, H-5′b and H-3″b), 3.71 (1H, dd, *J* 1.8 and 9.7, H-4′), 3.50 (1H, m, H-3″b), 2.21–2.08 (2H, m, H-1″a and H-2″a), 1.99 (1H, m, H-2″b), 1.87 (1H, m, H-1″b), 1.11–0.94 (28H, m, –$SiCH(CH_3)_2$); δ_C ($CDCl_3$) 164.7 (C-4), 152.3 (C-2), 140.8 (C-6), 101.6 (C-5), 88.1 (C-1′), 80.9 (C-4′), 78.4 (C-2′), 73.1 (C-3′), 62.9 (C-3″), 60.2 (C-5′), 31.4 (C-2″), 25.9 (C-1″), 17.5, 17.4, 17.2, 17.1, 17.0, 16.8 and 16.7 ($SiCH(CH_3)_2$), 13.5, 13.1, 12.9 and 12.4 ($SiCH(CH_3)_2$); FAB-MS *m*/*z* 545 [M + H]$^+$.

1-[2-*C*-(3-Methanesulfonyloxypropyl)-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]uracil (19)

By the procedure described above for synthesis of compound **7a**, mesylation of nucleoside **18** (1.43 g, 2.63 mmol) using methanesulfonyl chloride (0.31 cm^3, 3.94 mmol) in pyridine (10 cm^3) afforded a residue that was purified by dry column chromatography[33] [1–3% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **19** as a white solid material (1.22g, 74%); R_f 0.64 (CH_2Cl_2–MeOH 92 : 8, v/v); δ_H ($CDCl_3$) 7.86 (1H, d, *J* 8.1, H-6), 5.79 (1H, s, H-1′), 5.68 (1H, d, *J* 8.1, H-5), 4.34–4.31 (2H, m, H-3″), 4.21 (1H, d, *J* 9.2, H-3′), 4.14 (1H, dd, *J* 1.5 and 13.5 Hz, H-5′a), 3.99 (1H, dd, *J* 2.8 and 13.4, H-5′b), 3.79 (1H, d, *J* 9.2, H-4′), 3.03 (3H, s, –SO_2CH_3), 2.16–2.13 (3H, m, H-2″ and H-1″a), 1.82 (1H, m, H-1″b), 1.11–0.97 (28H, m, –$SiCH(CH_3)_2$); δ_C($CDCl_3$) 163.7 (C-4), 151.5 (C-2), 140.6 (C-6), 101.6 (C-5), 88.4 (C-1′), 81.2 (C-4′), 79.9 (C-2′), 74.0 (C-3′), 70.5 (C-3″),

60.2 (C-5′), 37.4 (–SO_2CH_3), 29.6 (C-1″), 22.8 (C-2″), 17.5, 17.4, 17.2, 17.1, 17.0 and 16.9 (–SiCH(CH_3)$_2$), 13.5, 13.1, 12.9 and 12.5 (–Si$CH(CH_3)_2$); FAB-MS *m*/*z* 623 $[M + H]^+$.

1-[2-*O*,2-*C*-Propano-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*β*-D-arabinofuranosyl]uracil (20)

By the procedure described above for synthesis of compound **8a**, cyclization of nucleoside **19** (1.20 g, 1.93 mmol) using NaH (60% suspension in mineral oil, 154 mg, 3.85 mmol) in anhydrous THF (15.0 cm^3) afforded a residue that was purified by dry column chromatography[33] [0–2% (v/v) MeOH in CH_2Cl_2] to furnish nucleoside **20** as a white solid material (772 mg, 76%); R_f 0.52 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.18 (1H, br s, –NH), 7.80 (1H, d, *J* 8.2, H-6), 5.76 (1H, s, H-1′), 5.67 (1H, dd, *J* 1.8 and 8.1, H-5), 4.22 (1H, d, *J* 9.2, H-3′), 4.12 (1H, dd, *J* 1.6 and 13.2, H-5′a), 3.98 (1H, dd, *J* 2.8 and 13.2, H-5′b), 3.77–3.74 (2H, m, H-3″), 3.65 (1H, ddd, *J* 1.7, 2.6 and 9.3, H-4′), 2.41 (1H, m, H-1″a), 2.14 (1H, m, H-2″a), 1.99–1.86 (2H, m, H-1″b and H-2″b), 1.11–0.94 (28H, m, –SiCH(CH_3)$_2$); δ_C ($CDCl_3$) 163.4 (C-4), 150.4 (C-2), 140.1 (C-6), 101.2 (C-5), 88.8 and 88.5 (C-1′ and C-2′), 80.9 (C-4′), 71.0 (C-3′), 69.7 (C-3″), 60.1 (C-5′), 29.3 (C-1″), 25.7 (C-2″), 17.4, 17.3, 17.2, 17.1, 17.0 and 16.9 (–SiCH(CH_3)$_2$), 13.4, 13.0, 12.8 and 12.4 (–Si$CH(CH_3)_2$); FAB-MS *m*/*z* 527 $[M + H]^+$; Anal. Calcd for $C_{24}H_{42}N_2O_7Si_2$: C, 54.7; H, 8.0; N, 5.3. Found: C, 54.6; H, 8.1; N, 5.0%.

1-(2-*O*,2-*C*-Propano-*β*-D-arabinofuranosyl)uracil (21)

Compound **20** (734 mg, 1.4 mmol) was coevaporated with anhydrous acetonitrile (2 × 3 cm^3), dissolved in anhydrous THF (10 cm^3) and Et_3N,3HF (0.45 cm^3, 2.79 mmol) was added, and the mixture was stirred for 2 h at rt. The mixture was then evaporated to dryness under reduced pressure, and the residue was purified by column chromatography [3–6% (v/v) MeOH in CH_2Cl_2] affording nucleoside **21** as a white solid material (361 mg, 91%); R_f 0.19 (CH_2Cl_2–MeOH 92 : 8, v/v); δ_H (pyridine-d_5) 13.33 (1H, br s, –NH), 8.45 (1H, d, *J* 8.1, H-6), 6.57 (1H, s, H-1′), 5.84 (1H, d, *J* 7.9, H-5), 4.78 (1H, d, *J* 5.4, H-3′), 4.37–4.26 (3H, m, H-4′ and H-5′), 3.81–3.72 (2H, m, H-3″), 2.78 (1H, m, H-1″a), 2.16 (1H, m, H-1″b), 1.97 (1H, m, H-2″a), 1.86 (1H, m, H-2″b); δ_C (pyridine-d_5) 164.6 (C-4), 152.6 (C-2), 142.7 (C-6), 101.7 (C-5), 91.6 (C-2′), 89.0 (C-1′), 85.3 (C-4′), 74.3 (C-3′), 70.0 (C-3″), 61.5 (C-5′), 29.5 (C-1″), 26.4 (C-2″); FAB-MS *m*/*z* 285 $[M + H]^+$; Anal. Calcd for $C_{12}H_{16}N_2O_6$: C, 50.7; H, 5.7; N, 9.9. Found: C, 50.3; H, 5.8; N, 9.6%.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,2-*C*-propano-*β*-D-arabinofuranosyl]uracil (22)

By the procedure described above for synthesis of compound **4**, compound **21** was dimethoxytritylated (260 mg, 0.91 mmol) using 4,4′-dimethoxytrityl chloride (465 mg, 1.37 mmol) in anhydrous pyridine (5 cm^3) affording a yellow oil that was purified by dry column chromatography[33] [0–2% MeOH in CH_2Cl_2, containing 0.5% of pyridine (v/v/v)] to yield nucleoside **22** as a white solid material (536 mg, ~100%) R_f 0.45 (CH_2Cl_2–MeOH 92 : 8, v/v); δ_H ($CDCl_3$) 9.03 (1H, br s, –NH), 7.82 (1H, d, *J* 8.2, H-6), 7.35–7.08 (9H, m, ArH), 6.77–6.74 (4H, m, ArH), 5.87 (1H, s, H-1′), 5.35 (1H, dd, *J* 2.0 and 8.1, H-5), 4.14 (1H, d, *J* 7.0, H-3′), 3.76 (1H, m, H-3″a), 3.71–3.68 (7H, m, 2 × –OCH_3 and H-4′), 3.60 (1H, m, H-3″b), 3.44–3.41 (2H, m, H-5′), 2.24 (1H, m, H-1″a), 1.90 (1H, m, H-2″a), 1.84–1.80 (2H, m, H-1″b and H-2″b); δ_C ($CDCl_3$) 163.3 (C-4), 158.5, 150.5 (C-2), 144.4, 141.5 (C-6), 136.0, 135.4, 130.0, 129.9, 128.1, 128.0, 127.8, 126.9, 125.1, 113.1, 101.2 (C-5), 89.7 and 88.0 (C-1′ and C-2′), 86.7 (–CPh_3), 81.4 (C-4′), 73.2 (C-3′), 69.5 (C-3″), 61.7 (C-5′), 55.1 (2 × –OCH_3), 28.4 (C-1″), 25.5 (C-2″); FAB-MS *m*/*z* 587 $[M + H]^+$ and 586 $[M]^+$.

1-[3-*O*-(2-Cyanoethoxy)(diisopropylamino)phosphino-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,2-*C*-propano-*β*-D-arabinofuranosyl]uracil (23)

By the procedure described above for synthesis of compound **10**, treatment of compound **22** (340 mg, 0.59 mmol) with 2-cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (166 mg, 0.70 mmol) in the presence of *N*,*N*′-diisopropylethylamine (0.4 cm^3) and anhydrous CH_2Cl_2 (3.0 cm^3) afforded a residue that was purified by column chromatography [EtOAc–*n*-hexane–Et_3N (49.5 : 50 : 0.5 (v/v/v)] to yield phosphoramidite **23** (277 mg, 61%) as a white solid material.. R_f 0.61 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_P ($CDCl_3$) 152.2 and 151.3; FAB-MS *m*/*z* 787 $[M + H]^+$.

1-[2-*C*-(2-Hydroxyethyl)-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*β*-D-arabinofuranosyl]uracil (24)

By the procedure described above for synthesis of compound **11a**, oxidative cleavage of the terminal double bond of nucleoside **17** (4.83 g, 9.18 mmol) using osmium tetraoxide (1.0 cm^3 of a 2.5% solution in *tert*-butyl alcohol, 98 μmol) and sodium periodate (7.52 g, 35.2 mmol) in a mixture of THF (25.0 cm^3) and H_2O (25.0 cm^3), followed by reduction with sodium borohydride (1.04 g, 27.5 mmol), afforded a residue that was purified by column chromatography [3% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **24** as a white solid material (2.27 g, 47%); R_f 0.47 (CH_2Cl_2–MeOH 92 : 8, v/v); δ_H ($CDCl_3$) 11.23 (1H, br s, –NH), 7.91 (1H, d, *J* 8.1, H-6), 6.01 (1H, s, H-1′), 5.71 (1H, dd, *J* 2.2 and 8.2, H-5), 4.61 (1H, m, H-2″a), 4.17–3.94 (4H, m, H-2″b, H-3′, H-5′), 3.66 (1H, dd, *J* 1.8 and 9.9, H-4′), 2.50 (1H, m, H-1″a), 1.60 (1H, m, H-1″b), 1.11–0.94 (28H, m, –SiCH-(CH_3)$_2$), δ_C ($CDCl_3$) 164.5 (C-4), 152.5 (C-2), 140.2 (C-6), 101.8 (C-5), 87.4 (C-1′), 80.9 (C-2′), 80.1 (C-4′), 73.1 (C-3′), 59.9 (C-2″), 58.5 (C-5′), 30.3 (C-1″), 17.5, 17.4, 17.3, 17.2, 17.1, 17.0, 16.8 and 16.7 (–SiCH(CH_3)$_2$), 13.4, 13.0, 12.8 and 12.3 (–Si$CH(CH_3)_2$); FAB-MS *m*/*z* 531 $[M + H]^+$.

1-[2-*C*-(2-Methanesulfonyloxyethyl)-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*β*-D-arabinofuranosyl]uracil (25)

By the procedure described above for synthesis of compound **7a**, mesylation of nucleoside **24** (1.68 g, 3.17 mmol) using methanesulfonyl chloride (0.74 cm^3, 9.5 mmol) in the presence of pyridine (20 cm^3) afforded nucleoside **25** as a white solid material which was used without further purification in the next step; R_f 0.66 (CH_2Cl_2–MeOH 92 : 8, v/v); δ_H ($CDCl_3$) 9.82 (1H, br s, –NH), 7.80 (1H, d, *J* 8.1, H-6), 5.86 (1H, s, H-1′), 5.66 (1H, d, *J* 8.2, H-5), 4.75 (1H, m, H-2″a), 4.62 (1H, m, H-2″b), 4.22 (1H, d, *J* 9.0, H-3′), 4.14 (1H, dd, *J* 1.8 and 13.4, H-5′a), 4.00 (1H, dd, *J* 2.7 and 13.4, H-5′b), 3.81 (1H, m, H-4′), 3.05 (3H, s, –SO_2CH_3), 2.53 (1H, m, H-1″a), 2.25 (1H, m, H-1″b), 1.11–0.97 (28H, m, –SiCH(CH_3)$_2$); δ_C ($CDCl_3$) 163.6 (C-4), 151.4 (C-2), 140.4 (C-6), 101.6 (C-5), 88.1 (C-1′), 81.0 (C-4′), 79.5 (C-2′), 74.3 (C-3′), 66.3 (C-2″), 60.1 (C-5′), 37.2 (–SO_2CH_3), 33.4 (C-3″), 17.4, 17.3, 17.2, 17.1, 17.0, 16.9, 16.8 and 16.7 (–SiCH-(CH_3)$_2$), 13.4, 12.9, 12.8 and 12.4 (–Si$CH(CH_3)_2$); FAB-MS *m*/*z* 509 $[M + H]^+$.

1-[2-*O*,2-*C*-Ethano-3,5-di-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-*β*-D-arabinofuranosyl]uracil (26)

By the procedure described above for synthesis of compound **8a**, cyclization of nucleoside **25** (1.54 g, 2.53 mmol) using NaH (60% suspension in mineral oil, 404 mg, 10.1 mmol) in anhydrous THF (20.0 cm^3) afforded a residue that was purified by anhydrous column chromatography [1–3% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **26** as a white solid material (704 mg, 54%); R_f 0.52 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.05 (1H, br s, NH), 7.61 (1H, d, *J* 8.1, H-6), 6.20 (1H, s, H-1′), 5.66 (1H, d, *J* 2.1 and 8.1, H-5), 4.59 (1H, ddd, *J* 5.3, 7.3 and 8.2, H-2″a), 4.42 (1H, m, H-2″b), 4.23–4.09 (2H, m, H-3′ and

Table 2 MALDI-MS m/z $[M - H]^-$

	Ribo series		Ara series	
	Found	Calcd	Found	Calcd
ON1: T_7(**X5**)T_6	4250	4252	4234	4238
ON2: T_7(**X4**)T_6	4240	4238	4224	4224
ON3: T_5(**X5**)$_4T_5$	4419	4420	4359	4364
ON4: T_5(**X4**)$_4T_5$	4367	4364	4303	4308
ON6: (**X5**)$_9$T	3484	3484	3356	3358
ON7: (**X4**)$_9$T	3361	3358	3229	3232
ON9: G(**X5**)GA(**X5**)A(**X5**)GC	2922	2921	2876	2879
ON10: G(**X4**)GA(**X4**)A(**X4**)GC	2881	2879	2846	2837

H-5′a), 3.97 (1H, dd, *J* 2.7 and 13.2, H-5′b), 3.55 (1H, ddd, *J* 1.7, 2.6 and 9.4, H-4′), 3.23 (1H, m, H-1″a), 2.57 (1H, m, H-1″b), 1.11–0.96 (28H, m, –SiCH(CH_3)$_2$); δ_C ($CDCl_3$) 163.3 (C-4), 150.8 (C-2), 139.7 (C-6), 101.7 (C-5), 90.1 (C-2′), 87.9 (C-1′), 79.1 (C-4′), 72.2 (C-3′), 67.9 (C-2″), 60.0 (C-5′), 24.4 (C-1″), 17.5, 17.4, 17.2, 17.1, 17.0 and 16.9 (SiCH(CH_3)$_2$), 13.6, 12.9, 12.8 and 12.5 (SiCH(CH_3)$_2$); FAB-MS m/z 513 $[M + H]^+$; Anal. Calcd for $C_{23}H_{40}N_2O_7Si_2$: C, 53.9; H, 7.9; N, 5.5. Found: C, 53.5; H, 7.9; N, 5.3%.

1-(2-*O*,2-*C*-Ethano-*β*-D-arabinofuranosyl]uracil (27)

By the procedure described above for synthesis of compound **21**, desilylation of nucleoside **26** (103 mg, 0.195 mmol) using $Et_3N{\cdot}3HF$ (0.1 cm^3, 0.61 mmol) in THF (3.0 cm^3) afforded a residue that was purified by dry column chromatography [33] [3–8% (v/v) MeOH in CH_2Cl_2] to yield nucleoside **27** as a white solid material which was used in the next step without further purification (45 mg, 82%); R_f 0.20 (CH_2Cl_2–MeOH 98 : 2, v/v); δ_H (pyridine-d_5) 13.43 (1H, br s, NH), 8.32 (1H, d, *J* 8.1, H-6), 6.86 (1H, s, H-1′), 5.83 (1H, d, *J* 8.1, H-5), 4.88 (1H, d, *J* 6.1, H-3′), 4.55 (1H, ddd, *J* 5.5, 7.2 and 8.5, H-2″a), 4.44 (1H, m, H-2″b), 4.33–4.22 (3H, m, H-4′, H-5′), 3.45 (1H, ddd, *J* 7.2, 8.9 and 11.3, H-1″a), 2.79 (1H, ddd, *J* 6.0, 8.5 and 11.4, H-1″b); δ_C (pyridine-d_5) 164.8 (C-4), 152.9 (C-2), 142.5 (C-6), 101.9 (C-5), 92.7 (C-2′), 88.6 (C-1′), 83.9 (C-4′), 74.6 (C-3′), 68.0 (C-2″), 61.3 (C-5′), 25.0 (C-1″); FAB-MS m/z 271 $[M + H]^+$.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2-*O*,2-*C*-ethano-*β*-D-arabinofuranosyl]uracil (28)

By the procedure described above for synthesis of compound **4**, dimethoxytritylation of nucleoside **27** (101 mg, 0.37 mmol) using 4,4′-dimethoxytrityl chloride (190 mg, 0.56 mmol) in anhydrous pyridine (5 cm^3) afforded a yellow oil that was purified by dry column chromatography [33] [0–3% MeOH in CH_2Cl_2, containing 0.5% of pyridine (v/v/v)] to yield nucleoside **28** as a white solid material (190 mg, 89%); R_f 0.26 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_H ($CDCl_3$) 9.22 (1H, br s, NH), 7.71 (1H, d, *J* 8.1, H-6), 7.33–7.07 (9H, m, ArH), 6.77–6.74 (4H, m, ArH), 6.19 (1H, s, H-1′), 5.37 (1H, d, *J* 8.2, H-5), 4.42–4.32 (2H, m, H-2″), 4.19 (1H, d, *J* 7.3, H-3′), 3.71–3.67 (7H, m, 2 × –OCH_3 and H-4′), 3.43 (1H, dd, *J* 3.3 and 10.8, H-5′a), 3.35 (1H, dd, *J* 4.0 and 11.0 Hz, H-5′b), 3.06 (1H, m, H-1″a), 2.48 (1H, m, H-1″b); δ_C ($CDCl_3$) 163.3 (C-4), 158.5, 150.8 (C-2), 144.3, 141.1 (C-6), 136.1, 135.3, 130.0, 129.0, 128.9, 128.1, 128.0, 126.9, 125.1 and 113.1, 101.4 (C-5), 90.9 (C-2′), 87.5 (C-1′), 86.7 (Ar_3C), 80.1 (C-4′), 73.8 (C-3′), 67.7 (C-2″), 61.6 (C-5′), 55.1 (2 × –OCH_3), 23.8 (C-1″); FAB-MS m/z 573 $[M + H]^+$ and 572 $[M]^+$.

1-[3-*O*-(2-Cyanoethoxy)(diisopropylamino)phosphino-5-*O*-(4,4′-dimethoxytrityl)-2-*O*,2-*C*-ethano-*β*-D-arabinofuranosyl]uracil (29)

By the procedure described above for synthesis of compound **10**, treatment of compound **28** (310 mg, 0.54 mmol) with 2-cyanoethyl *N*,*N*′-diisopropylphosphoramidochloridite (0.36 cm^3, 1.62 mmol) in the presence of *N*,*N*′-diisopropylethylamine (1.0 cm^3) and anhydrous CH_2Cl_2 (5.0 cm^3) afforded a residue that was purified by column chromatography [EtOAc–*n*-hexane–Et_3N (49.5 : 50 : 0.5 (v/v/v)] to yield the phosphoramidite **29** (251 mg, 60%) as a white solid material; R_f 0.60 (CH_2Cl_2–MeOH 95 : 5, v/v); δ_P ($CDCl_3$) 152.6 and 152.0; FAB-MS m/z 773 $[M + H]^+$.

Synthesis, deprotection and purification of oligonucleotides

All oligomers **ON1**–**ON11** were prepared using the phosphoramidite approach on a Biosearch 8750 DNA synthesizer at 0.2 μmol scale on CPG solid supports (BioGenex). The stepwise coupling efficiencies of the phosphoramidites **10**, **15**, **23** and **29** (10 min coupling time) and of unmodified deoxynucleoside phosphoramidites were > 98% using 1*H*-tetrazole as activator. Standard conditions were used on the synthesizer except that "hand-coupling conditions" [34] were used when coupling the phosphoramidites **10**, **15**, **23** and **29**. After standard deprotection and cleavage from the solid support using 32% aqueous ammonia (12 h, 55 °C), the oligomers were purified by precipitation from ethanol. The composition of the oligomers was verified by MALDI-MS analysis and the purity (> 80%) by capillary gel electrophoresis. Table 2 shows the MALDI-MS data ($[M - H]^-$; found/calcd).

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 solution (10 mM sodium phosphate, 100 mM sodium chloride, 0.1 mM EDTA, pH 7.0). Concentrations of 1.5 μM of the two complementary strands were used assuming identical extinction coefficients for modified and unmodified oligonucleotides. The absorbance was monitored at 260 nm while raising the temperature at a rate of 1 °C per min. The melting temperatures (T_m values) of the duplexes were determined as the maximum of the first derivatives of the melting curves obtained.

Quantum mechanical calculation

All *ab initio* quantum mechanical calculations were carried out using the Gaussian 98 program.[35] To determine the potential energy of pseudorotation, sugar torsion angles ν_2 and ν_4 were calculated using the theory of Altona and Sundaralingam with a maximal puckering amplitude of 36°.[36] For each point along the pseudorotation pathway, full geometry optimizations (HF/6-31G) were carried out while maintaining the desired ν_2 and ν_4 angles and, in some cases, the ε torsion angle was constrained as well. Single point energies were determined using second-order Møller–Plesset theory (MP2) with the cc-pVTZ basis set.

Karplus equation

A Karplus relationship correlating $J_{H3'H4'}$ and the ν_3 torsion angle was constructed using a state-of-the-art Karplus equation (eqn. 1) for nucleosides and nucleotides developed by Altona

and co-workers,[28–30] where the electronegativity of the HCCH-fragment substituents is accounted for in the coefficients C_m and S_n:

$$J_{HH}(\theta) = \sum_{m=0}^{3} C_m \cos(m\theta) + \sum_{n=1}^{3} S_n \sin(n\theta) \qquad (1)$$

Force field calculations

Force field calculations were carried out using the AMBER7 suite of programs.[37] Atomic charges for the **X4-Ribo** and **X4-Ara** nucleotides were calculated using the RESP procedure.[38] Initial models of A- and B-type geometry were built using the *nucgen* module and the relevant residues were modified. The duplexes were solvated with a truncated octahedral box of TIP3P water molecules, so that no duplex atom was closer to the boundary than 10 Å, which resulted in truncated octahedral boxes with side lengths of ~58 Å. To obtain electrostatic neutrality, 16 sodium ions were included in the calculations. The total system sizes varied between 12000 and 13000 atoms. All bonds with hydrogen atoms involved were constrained with SHAKE. The particle mesh Ewald (PME) method was used in all calculations to evaluate electrostatic interactions. The default parameter values in AMBER were used in PME calculations. For equilibration, the systems were initially energy minimised, followed by 70 ps of molecular dynamics at 300 K and 1 atm pressure, in which the solute was positionally restrained with incrementally decreasing force constants (500 to 0.1 kcal mol^{-1} $Å^{-2}$). In the production calculations, temperature coupling was switched off and the simulations were carried out in the microcanonical ensemble. A time step of 2 fs was used. In the simulations starting from A-type duplex geometry, the trajectories were extended to 2 ns, while the B-simulations were run for 1 ns.

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