Synthesis and hybridization studies of α-configured arabino nucleic acids†

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Synthesis of α-L-*arabino*- and α-D-*arabino*-configured pentofuranosyl nucleosides of four of the natural bases [thymine (ara-T), adenine (ara-A), cytosine (ara-C) and guanine (ara-G)] is reported together with hybridization properties of oligonucleotides containing α-L-ara-T and -A, α-D-ara-T and -A, and 2′-amino-α-L-ara-T monomers. 2′-*O*-Acetylated α-L-ara-T, -A, -C and -G, α-D-ara-T, -A, -C and -G, and N2′-acylated-α-L-ara-T phosphoramidite building blocks were synthesized and used together with standard DNA phosphoramidites for solid-phase synthesis of 18-mer oligonucleotides. Thermal denaturation experiments showed that incorporation of three or six of the *arabino*-configured monomers into DNA-oligonucleotides reduced the binding affinity towards antiparallel DNA/RNA complements. Fully modified α-L-ara-oligonucleotides did not hybridize with DNA/RNA complements, whereas hybridization of fully modified α-D-ara-oligonucleotides with complementary DNA/RNA in parallel strand orientation was confirmed.

Introduction

To understand why the nucleic acids in Nature are based on β-D-pentofuranosyl ribonucleotide building blocks, numerous alternative nucleic acid systems have been studied, including ones based on pentopyranosyl or hexopyranosyl nucleotide building blocks.[1–10] Eschenmoser and co-workers have found that pentopyranosyl-(4′→2′)-oligonucleotide systems composed of β-ribopyranosyl, β-xylopyranosyl, α-lyxopyranosyl or α-arabinopyranosyl units as repeating sugar building blocks constitute stronger Watson–Crick base pairing systems than RNA.[9–12] The α-arabinopyranosyl system in fact belongs to the strongest oligonucleotide base pairing systems known,[12] which signifies that maximization of base pairing strength within the domain of pentose-derived oligonucleotide systems was not the decisive driving force during the evolution of RNA.

Oligonucleotides (ONs) involving pentofuranosyl-based systems, *i.e.* RNA stereoisomers with β-L-*ribo*-,[13] α-D-*ribo*-,[14] α-L-*ribo*-,[15–17] β-D-*arabino*-,[18–20] and α-D-*arabino*-[21,22] configured nucleotide monomers have also been studied (Fig. 1).

Ashley[13] did not observe any hybridization between β-L-*ribo* ON L-r$(U)_{12}$ and complementary DNA. In contrast, a mixture of L-r$(U)_{12}$ and complementary RNA displayed a sharp hyperchromic transition upon thermal heating indicative of hybridization at room temperature. For α-D-RNA,[14] hybridization was not significant between a fully modified α-D-dodecaribonucleotide and DNA in antiparallel orientation, whereas a clear transition was observed in parallel orientation. These results were in accordance with earlier reports for α-D-DNA,[23–25] which showed binding with complementary DNA in parallel orientation. A special aspect of the α-anomeric ONs α-DNA and α-RNA[14] is their high resistance towards nucleases.[26,27] Interestingly, Sawai *et al.*[28] showed by CD studies that single stranded α-DNA displays weaker CD bands than those of corresponding β-oligomers, which indicates weaker base-stacking interactions of the α-oligomers. Incorporation of a single α-L-RNA[15] monomer into a 9-mer DNA strand induced a hybridization preference towards complementary RNA as compared to DNA, a trend that was also observed upon incorporation of three α-L-RNA monomers.

The 2′-epimer of RNA, β-D-arabinofuranosyl nucleic acid (ANA),[18–20] exhibits preferential binding towards complementary RNA over DNA, albeit in both cases with lower binding affinity than the corresponding unmodified duplexes.[26] Important for potential antisense applications are the facts that ANA oligomers are resistant to 3′-exonucleases and that ANA/RNA duplexes are substrates for the enzyme RNase H.[26] Oligomers composed of

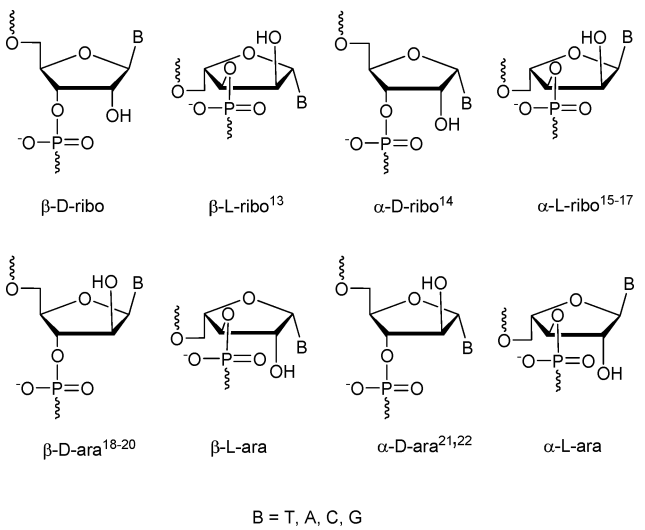

Fig. 1 Stereoisomers of RNA having *ribo*- and *arabino*-configuration.

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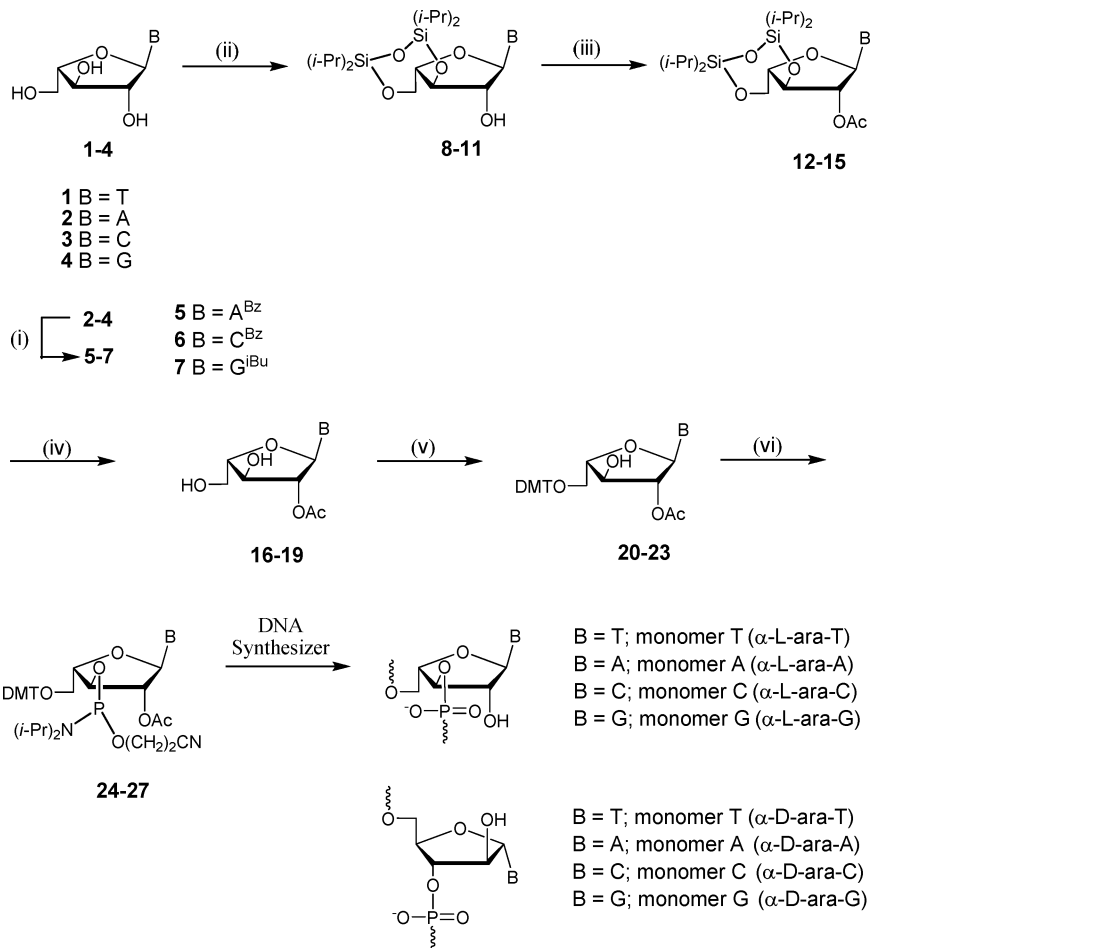
monomers all having α-D-*arabino* configuration have also been prepared. Such α-D-*ara* ONs preferentially form duplexes of parallel strand orientation with DNA complements, but antiparallel duplexes with α-D-ara ON complements.[21,22] A pentadeca 1-(α-D-arabinofuranosyl)thymine oligomer displayed stronger RNA than DNA binding and also the capability to form a triplex with a β-DNA duplex.[22] Notably, the stability induced by an α-D-ara nucleotide monomer against exonucleolytic degradation was found to be significant.[22] A recent report on a potentially prebiotic synthesis of pyrimidine β-D-ribonucleosides by photochemically induced anomerization of α-D-ribonucleosides has highlighted the relevance of studying α-configured nucleotides as possible components of prebiotic nucleic acids.[29]

As illustrated by the selected literature results described above, several *ribo*- and *arabino*-configured pentofuranosyl stereoisomers of RNA have been synthesized and studied. To expand these studies, we herein report synthesis of α-L-*arabino*- and α-D-*arabino*-configured nucleosides of four of the natural bases, oligomers thereof, and binding affinities of these towards DNA, RNA and α-*arabino*-configured ONs. This study extends earlier reports on α-D-ara ONs, and is the first study of α-L-ara ONs. We report on fully α-D/L-ara modified ONs as well as stereoirregular ONs, *i.e.* duplexes containing α-L-*arabino*-, α-D-*arabino*- and β-D-*ribo*-configured nucleotide monomers.

Results and discussion

Synthesis

Nucleosides **1–4** (α-L-ara nucleosides) were synthesized from L-arabinose by a known procedure.[30] The nucleobase amino groups of nucleosides **2–4** were acylated using the transient protection method[31] to give novel nucleosides **5** (76%) and **7** (78%), and the known nucleoside **6**[32] (92%). Regioselective O3′- and O5′-protection of the nucleosides **1** and **5–7** was performed using 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane ($TIPDSCl_2$) in the presence of pyridine furnishing nucleosides **8**[33] (80%), **9** (81%), **10** (66%) and **11** (76%) which were next O2′-acetylated by acetic anhydride in pyridine to afford nucleoside **12** (86%), **13** (90%), **14** (88%) and **15** (80%), respectively. Removal of the TIPDS group from nucleosides **12–15** by $Et_3N{\cdot}3HF$ in acetonitrile yielded O2′-acylated nucleosides **16** (91%), **17** (93%), **18** (80%) and **19** (79%). Subsequent reaction with 4,4′-dimethoxytrityl chloride (DMTCl) in pyridine selectively afforded the O5′-DMT protected nucleosides **20** (84%), **21** (78%), **22** (81%) and **23** (45%), which were O3′-phoshphitylated by reaction with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite to give the target nucleoside phosphoramidite derivatives **24** (78%), **25** (79%), **26** (80%) and **27** (45%) (Scheme 1), respectively, suitable for automated solid phase incorporation of α-L-*ara* monomers into ONs.



Scheme 1 *Reagents and conditions*: i) TMSCl, pyridine, BzCl; then aq. NH_3 (for compounds **2–3**); TMSCl, pyridine, isobutyric anhydride; then aq. NH_3 (for compound **4**); ii) $TIPDSCl_2$, pyridine; iii) Ac_2O, pyridine; iv) $Et_3N{\cdot}3HF$, MeCN; v) DMTCl, pyridine, CH_2Cl_2; vi) $NCCH_2CH_2OP(Cl)N(i\text{-}Pr)_2$, DIPEA, CH_2Cl_2.

The enantiomeric α-D-ara nucleoside derivatives, *i.e.* eventually the α-D-ara-T, -A^{Bz}, -C^{Bz} and -G^{iBu} phosphoramidites corresponding to **24–27**, were synthesized from D-arabinose by identical protocols as described above for the α-L-ara nucleosides. It should be noted that we followed a different protocol than the one published by Henke and Pfleiderer.[21] In the experimental descriptions found in the ESI,† all α-D-ara nucleoside derivatives are denoted with the same number as the corresponding α-L-ara nucleoside derivatives but followed by "D" (*i.e.* 20 and 20D for the α-L-ara and α-D-ara nucleoside, respectively).

In order to evaluate a possible influence of an amino functionality at the 2′-position of these α-ara nucleoside derivatives on hybridization capability, we also synthesized α-L-ara phosphoramidite derivative **33** starting from α-L-arabinofuranosylthymine (**1**). Nucleoside **1** was converted to 2,2′-anhydro-α-L-ribofuranosylthymine (**28**) in 32% yield by reaction with diphenyl carbonate (DPC) in the presence of sodium bicarbonate as catalyst and anhydrous *N*,*N*′-dimethylformamide as solvent. The resulting nucleoside **28** was further refluxed with sodium azide in anhydrous *N*,*N*′-dimethylformamide at 150 °C for 9 h to yield 2′-azido-2′-deoxy-α-L-arabinofuranosylthymine (**29**) in 61% yield. The azido group of nucleoside **29** was reduced to an amino group by reaction with 10% Pd-C/H_2 to yield 2′-amino-2′-deoxy-α-L-arabinofuranosylthymine (**30**) in 82% yield. The 2′-amino group of nucleoside **30** was selectively protected using ethyl trifluoroacetate and 4-(*N*,*N*-dimethyl)aminopyridine in methanol at room temperature for 5 h to afford 1-(2′-(*N*-trifluoroacetyl)amino-α-L-arabinofuranosyl)thymine (**31**) in 87% yield. Dimethoxytritylation was done using dimethoxytrityl chloride and pyridine to afford the corresponding nucleoside **32** in 78% yield which on phosphitylation at the 3′-position by reaction with *bis*(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine and *N*,*N*-diisopropylammonium tetrazolide in anhydrous acetonitrile was converted into the target phosphoramidite monomer **33** in 90% yield (Scheme 2).

The oligomers (Table 1) used in this study were synthesized on an automated DNA synthesizer using the phosphoramidite approach (see experimental section for details).[34] The influence of the α-ara modifications on the thermal denaturation temperature (T_m values) of duplexes was studied towards DNA and RNA complements (Tables 2–6) by UV thermal denaturation experiments using a medium salt buffer (100 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.0) at a concentration of 1.0 μM of each strand. The increase in absorbance at 260 nm as a function of time was recorded while the temperature was raised linearly from 5–80 °C at a rate of 1 °C/min. We decided to study partly modified duplexes containing short segments of three or six α-arabino configured nucleosides, and fully modified strands. With this experimental design we enabled evaluation of both stereoregular (α-ara:α-ara) and stereoirregular (α-ara:β-D-ribo; α-D-ara:α-L-ara) hybridization. In all cases, the denaturation curves displayed sigmoidal monophasic transitions.

Table 1 Oligonucleotides included in this study with MALDI-MS data shown for ara nucleotide-modified oligonucleotides[a]

ONs	Mass found	Mass calcd.
ON1 5′-d(TTA TTA TTA TAA AAT TAT)		
ON2 5′-d(ATA ATT TTA TAA TAA TAA)		
ON3 5′-r(UUA UUA UUA UAA AAU UAU)		
ON4 5′-r(AUA AUU UUA UAA UAA UAA)		
ON5 5′-d(AAT AAT AAT ATT TTA ATA)		
ON6 5′-d(TAT TAA AAT ATT ATT ATT)		
ON7 5′-r(AAU AAU AAU AUU UUA AUA)		
ON8 5′-r(UAU UAA AAU AUU AUU AUU)		
ON9 5′-d(TTA TTA TT α-L-ara(**A TA**)A AAT TAT)	5530	5537
ON10 5′-d(TTA TTA α-L-ara(**TTA TAA**) AAT TAT)	5578	5581
ON11 5′-d(TTA TTA TT α-D-ara(**A TA**)A AAT TAT)	5532	5537
ON12 5′-d(TTA TTA α-D-ara(**TTA TAA**) AAT TAT)	5598	5581
ON13 5′-d(ATA ATT T α-L-ara(**TA T**)AA TAA TAA)	5547	5551
ON14 5′-d(ATA ATT α-L-ara(**TTA TAA**) TAA TAA)	5597	5599
ON15 5′-d(ATA ATT T α-D-ara(**TA T**)AA TAA TAA)	5552	5551
ON16 5′-d(ATA ATT α-D-ara(**TTA TAA**) TAA TAA)	5598	5599
ON17 5′-d(TTA TTA TT α-L-ara(**A T^N**A)A AAT TAT)	5525	5532
ON18 5′-d(TTA TTA α-L-ara(**$T^N$$T^N$A T^N**AA)AAT TAT)	5573	5578
ON19 5′-d(ATA ATT T α-L-ara(**T^NA T^N**)AA TAA TAA)	5542	5549
ON20 5′-d(ATA ATT α-L-ara(**$T^N$$T^N$A T^N**AA) TAA TAA)	5590	5596
ON21 5′-α-L-ara-(**TTA TTA TTA TAA AAT TAT**)	5775	5774
ON22 5′-α-L-ara-(**ATA ATT TTA TAA TAA TAA**)	5795	5792
ON23 5′-α-D-ara-(**TTA TTA TTA TAA AAT TAT**)	5773	5774
ON24 5′-α-D-ara-(**ATA ATT TTA TAA TAA TAA**)	5786	5792

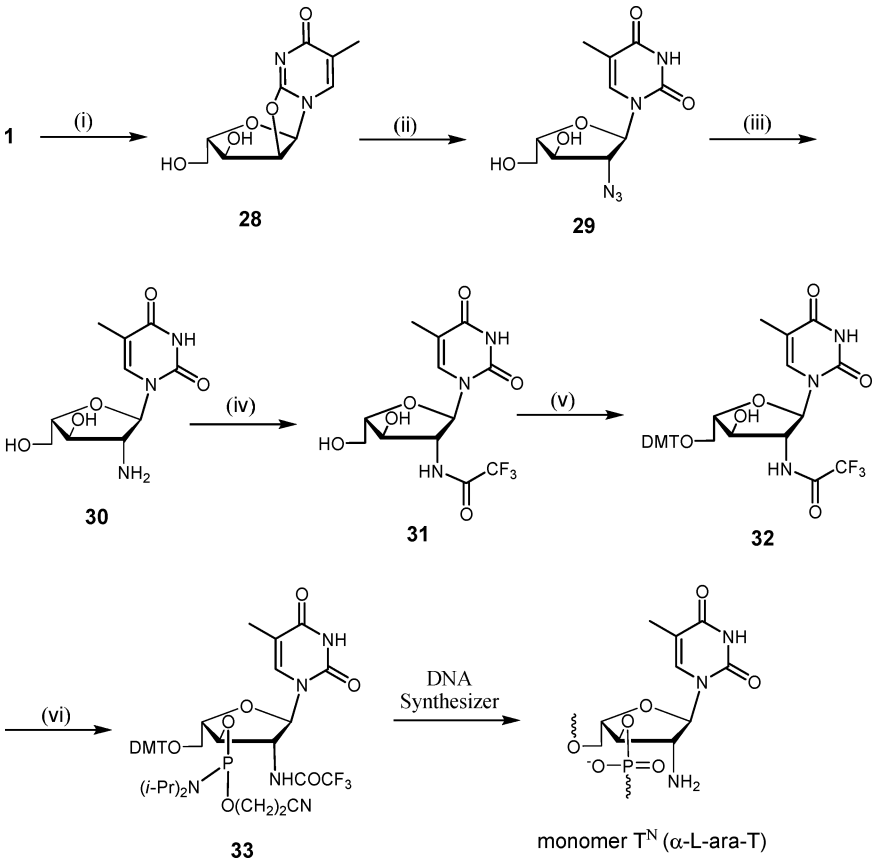
[a] A = adenin-9-yl DNA/RNA monomer, T = thymin-1-yl DNA monomer; U = uracil-1-yl RNA monomer; α-L-ara(···) indicates segments of the sequences that are α-L-ara nucleotides; α-D-ara(···) indicates segments of the sequences that are α-D-ara nucleotides; α-L-ara-T^N = 2′-amino-2′-deoxy-arabinofuranosyl thymin-1-yl monomer.

α-L-ara oligomers—hybridization in anti-parallel orientation

Table 2 displays hybridization studies between two strands that are complementary in an antiparallel binding mode. The first four entries show the T_m values recorded for the different unmodified duplexes containing DNA and/or RNA strands. These four T_m values are used below as reference values. In general, incorporation of three α-L-ara-T/A nucleotides in the middle of duplexes (entries 5–8), resulted in reduced thermal stabilities of the different duplexes by at least 1 °C per α-L-ara nucleotide. Accordingly, no duplex formation was observed when six α-L-ara-T/A nucleotides were incorporated in one of the strands (entries 9–12), nor for the fully modified α-L-ara oligonucleotides **ON21** and **ON22** (entries 13–16). The above data are based on Watson Crick base pairing between stereosiomeric nucleotides, *i.e.* α-L-ara and β-D-ribo (DNA/RNA) configured nucleotides. On the contrary, the duplexes of entries 17 and 18 include segments of three and six α-L-ara:α-L-ara base pairs, respectively. Interestingly, α-L-ara:α-L-ara base pairing stabilizes the duplexes relative to the duplexes having α-L-ara nucleotides in only one of the strands, which is confirmed by the T_m value of 21 °C for the fully α-L-ara modified duplex of entry 19. The latter result is in line with earlier data reported for fully α-D-ara modified antiparallel duplexes,[21] but it is noteworthy that duplexes having segments of both β-D-ribo:β-D-ribo base pairs and α-L-ara:α-L-ara base pairs (entries 17 and 18) display thermal stabilities comparable to those of duplexes formed between DNA/RNA strands (entries 1–4).

2′-Amino-2′-deoxy-α-L-ara oligomers—hybridization in anti-parallel orientation

To study the effect of 2′-amino-α-L-ara nucleotides we evaluated the duplexes depicted in Table 3 in the same sequence contexts as those in Table 2 (except for the fully modified α-L-ara oligomers).



Scheme 2 *Reagents and conditions*: i) Diphenyl carbonate, $NaHCO_3$, DMF; ii) NaN_3, DMF; iii) 10% Pd-C/H_2, MeOH; iv) $CF_3COOC_2H_5$, DMAP, MeOH; v) DMTCl, pyridine, CH_2Cl_2; vi) *bis*(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine, *N*,*N*-diisopropylammonium tetrazolide, MeCN.

We thus incorporated the α-L-ara-T^N monomer together with α-L-ara-T/A monomers, and obtained similar hybridization results as for the α-L-ara-T/A containing duplexes (Table 2). The duplexes containing **ON19** with an α-L-ara-(T^NAT^N) segment (entries 22 and 23) are thermally more stable than those containing **ON13** with an α-L-ara-(TAT) segment (entries 7 and 8). 2′-Amino derivatives of ONs are interesting as possible conjugation or protonation sites.[35,36] In the case of 2′-protonation one would expect more favorable T_m values for the α-L-ara-T^N containing oligomers than the α-L-ara-T containing monomers and we therefore evaluated hybridization at low salt conditions (as those described in the caption below Table 3 but with only 10 mM NaCl). Under these conditions, the duplexes of entries 22 and 28 in fact showed T_m values of 12.0 and 14.5 °C, respectively, whereas no transition was observed for the duplexes of entries 8 and 17. These results indicate that 2′-amino-α-L-ara nucleotides, contrary to 2′-amino-DNA nucleotides,[35] are at least in part protonated at pH 7. It is furthermore interesting that whereas 2′-amino-DNA nucleotides, relative to DNA nucleotides, have a detrimental effect on duplex stability,[35] 2′-amino-α-L-ara and α-L-ara nucleotides have a comparable effect.

α-D-ara oligomers—hybridization in anti-parallel orientation

In order to directly compare the effect of D- *vs.* L-stereochemistry, α-D-ara ONs were synthesized. Henke and Pfleiderer[21] have recently reported the synthesis and hybridization studies towards complementary DNA of α-D-ara ONs. They also studied fully modified and segment-modified α-D-ara oligomers and found that the presence of α-D-ara nucleotides was associated with sequence dependent decreases in T_m values. Similarly, we found (Table 4) that three α-D-ara-T/A nucleotides (entries 30–33) decreased T_m values relative to unmodified control duplexes (Table 2, entries 1, 3 and 4) to a similar extent as α-L-ara-T/A nucleotides (Table 2). Six α-D-ara-T/A nucleotides strongly reduced binding (entries 34–37) though stable duplexes were formed with complementary RNA, and no duplex formation was observed with the fully α-D-ara-T/A modified **ON23** and **ON24** (entries 38–41). As observed for the α-L-ara oligomers, α-D-ara:α-D-ara base pairing is more favorable than α-D-ara:β-D-ribo base pairing (entries 42–44), though it should be underlined that the duplexes displayed in entries 17 and 42 and in entries 18 and 43 are diastereoisomeric whereas those of entries 19 and 44 are enantiomeric.

α-D-ara oligomers—hybridization in parallel orientation

Fully modified α-L-ara or α-D-ara oligonucleotides (**ON21–ON24**) showed no binding to complementary DNA/RNA in antiparallel orientation. Earlier findings in the α-DNA series[23–25] suggest preferential duplex formation between α-configured ONs and DNA/RNA in parallel orientation. Similar results were

Table 2 α-L-ara oligomers—hybridization in anti-parallel orientation[a]

Entry	Duplexes	T_m(°C)
1.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	34.0
2.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	33.0
3.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	23.0
4.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	21.5
5.	**ON9** 5′-d(TTA TTA TTα-L-ara(**A TA**)A AAT TAT) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	21.0
6.	**ON9** 5′-d(TTA TTA TT α-L-ara(**A TA**)A AAT TAT) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	17.0
7.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON13** 3′-d(AAT AAT AA α-L-ara(**T AT**)T TTA ATA)	19.0
8.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON13** 3′-d(AAT AAT AA α-L-ara(**T AT**)T TTA ATA)	nt
9.	**ON10** 5′-d(TTA TTA α-L-ara(**TTA TAA**) AAT TAT) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	nt
10.	**ON10** 5′-d(TTA TTA α-L-ara(**TTA TAA**) AAT TAT) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	nt
11.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON14** 3′-d(AAT AAT α-L-ara(**AAT ATT**) TTA ATA)	nt
12.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON14** 3′-d(AAT AAT α-L-ara(**AAT ATT**) TTA ATA)	nt
13.	**ON21** 5′-α-L-ara(**TTA TTA TTA TAA AAT TAT**) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	nt
14.	**ON21** 5′-α-L-ara(**TTA TTA TTA TAA AAT TAT**) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	nt
15.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON22** 3′-α-L-ara(**AAT AAT AAT ATT TTA ATA**)	nt
16.	**ON13** 5′-r(UUA UUA UUA UAA AAU UAU) **ON22** 3′-α-L-ara(**AAT AAT AAT ATT TTA ATA**	nt
17.	**ON9** 5′-d(TTA TTA TT α-L-ara(**A TA**)A AAT TAT) **ON13** 3′-d(AAT AAT AA α-L-ara(**T AT**)T TTA ATA)	27.5
18.	**ON10** 5′-d(TTA TTA α-L-ara(**TTA TAA**) AAT TAT) **ON14** 3′-d(AAT AAT α-L-ara(**AAT ATT**) TTA ATA)	26.5
19.	**ON21** 5′-α-L-ara(**TTA TTA TTA TAA AAT TAT**) **ON22** 3′-α-L-ara(**AAT AAT AAT ATT TTA ATA**)	21.0

[a] See caption below Table 1. Thermal denaturation temperatures (T_m/°C) of unmodified or modified duplexes measured as the maximum of the first derivative of the melting curve (A_{260} *vs* temperature) recorded in medium salt buffer (100 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.0), using 1.0 μM concentration of the two complementary strands. T_m values are average of at least two measurements; "nt" denotes "no transition".

reported by Henke and Pfleiderer[21] for fully modified α-D-ara ONs which were shown to form parallel duplexes with complementary DNA of only slightly lower stabilities than the unmodified reference duplexes. We confirmed duplex formation in parallel orientation between fully modified α-D-ara ONs and complementary DNA (Table 5, entries 51 and 53) and also showed for the first time duplex formation between fully modified α-D-ara ONs and complementary RNA (entries 52 and 54). Parallel duplex formation neither between the unmodified reference DNA/RNA strands (entries 45–50) nor the fully modified α-L-ara ONs (**ON21** and **ON22**) and DNA/RNA were observed.

α-L-ara *vs* α-D-ara hybridization in anti-parallel orientation

We have shown above that α-D-ara:α-D-ara base pairing and α-L-ara:α-L-ara base pairing in central segments flanked by β-D-ribo:β-D-ribo base paired segments enable stable duplex

Table 3 2′-Amino-α-L-ara oligomers—hybridization in anti-parallel orientation[a]

Entry	Duplexes	T_m(°C)
20.	**ON17** 5′-d(TTA TTA TT α-L-ara(**A T^N**A)A AAT TAT) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	21.0
21.	**ON17** 5′-d(TTA TTA TT α-L-ara(**A T^N**A)A AAT TAT) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	15.5
22.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON19** 3′-d(AAT AAT AA α-L-ara(**T^N**AT^N)T TTA ATA)	23.0
23.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON19** 3′-d(AAT AAT AA α-L-ara(**T^N**AT^N)T TTA ATA)	14.0
24.	**ON18** 5′-d(TTA TTA α-L-ara(**T^N**T^{N}A T^{N}AA) AAT TAT) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	nt
25.	**ON18** 5′-d(TTA TTA α-L-ara(**T^N**T^{N}A T^{N}AA) AAT TAT) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	nt
26.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON20** 3′-d(AAT AAT α-L-ara(**AAT^N** AT^{N}T^N) TTA ATA)	nt
27.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON20** 3′-d(AAT AAT α-L-ara(**AAT^N** AT^{N}T^N) TTA ATA)	nt
28.	**ON17** 5′-d(TTA TTA TT α-L-ara(**A T^N**A)A AAT TAT) **ON19** 3′-d(AAT AAT AA α-L-ara(**T^N**AT^N)T TTA ATA)	26.0
29.	**ON18** 5′-d(TTA TTA α-L-ara(**T^N**T^{N}A T^{N}AA) AAT TAT) **ON20** 3′-d(AAT AAT α-L-ara(**AAT^N** AT^{N}T^N)TTA ATA)	21.0

[a] See captions below Table 1. Thermal denaturation temperatures (T_m/°C) of unmodified or modified duplexes measured as the maximum of the first derivative of the melting curve (A_{260} *vs* temperature) recorded in medium salt buffer (110 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, 5 mM Na_2HPO_4, pH 7.0), using 1.0 μM concentration of the two complementary strands. T_m values are average of at least two measurements; "nt" denotes "no transition".

Table 4 α-D-ara oligomers—hybridization in anti-parallel orientation[a]

Entry	Duplexes	T_m(°C)
30.	**ON11** 5′-d(TTA TTA TTα-D-ara(**A TA**)A AAT TAT) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	21.5
31.	**ON11** 5′-d(TTA TTA TTα-D-ara(**A TA**)A AAT TAT) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	21.0
32.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON15** 3′-d(AAT AAT AAα-D-ara(**T AT**)T TTA ATA)	19.0
33.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON15** 3′-d(AAT AAT AAα-D-ara(**T AT**)T TTA ATA)	17.5
34.	**ON12** 5′-d(TTA TTA α-D-ara(**TTA TAA**) AAT TAT) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	nt
35.	**ON12** 5′-d(TTA TTA α-D-ara(**TTA TAA**) AAT TAT) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	13.0
36.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON16** 3′-d(AAT AAT α-D-ara(**AAT ATT**) TTA ATA)	nt
37.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON16** 3′-d(AAT AAT α-D-ara(**AAT ATT**) TTA ATA)	16.0
38.	**ON23** 5′-α-D-ara(**TTA TTA TTA TAA AAT TAT**) **ON2** 3′-d(AAT AAT AAT ATT TTA ATA)	nt
39.	**ON23** 5′-α-D-ara(**TTA TTA TTA TAA AAT TAT**) **ON4** 3′-r(AAU AAU AAU AUU UUA AUA)	nt
40.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON24** 3′-α-D-ara(**AAT AAT AAT ATT TTA ATA**)	nt
41.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON24** 3′-α-D-ara(**AAT AAT AAT ATT TTA ATA**)	nt
42.	**ON11** 5′-d(TTA TTA TTα-D-ara(**A TA**)A AAT TAT) **ON15** 3′-d(AAT AAT AAα-D-ara(**T AT**)T TTA ATA)	26.5
43.	**ON12** 5′-d(TTA TTA α-D-ara(**TTA TAA**) AAT TAT) **ON16** 3′-d(AAT AAT α-D-ara(**AAT ATT**) TTA ATA)	16.0
44.	**ON23** 5′-α-D-ara(**TTA TTA TTA TAA AAT TAT**) **ON24** 3′-α-D-ara(**AAT AAT AAT ATT TTA ATA**)	20.0

[a] See captions below Table 1 and Table 2.

Table 5 α-D-ara oligomers—hybridization in parallel orientation[a]

Entry	Duplexes	T_m(°C)
45.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON5** 5′-d(AAT AAT AAT ATT TTA ATA)	nt
46.	**ON2** 5′-d(ATA ATT TTA TAA TAA TAA) **ON6** 5′-d(TAT TAA AAT ATT ATT ATT)	nt
47.	**ON3** 5′-r(UUA UUA UUA UAA AAU UAU) **ON7** 5′-r(AAU AAU AAU AUU UUA AUA)	nt
48.	**ON4** 5′-r(AUA AUU UUA UAA UAA UAA) **ON8** 5′-r(UAU UAA AAU AUU AUU AUU)	nt
49.	**ON1** 5′-d(TTA TTA TTA TAA AAT TAT) **ON7** 5′-r(AAU AAU AAU AUU UUA AUA)	nt
50.	**ON2** 5′-d(ATA ATT TTA TAA TAA TAA) **ON8** 5′-r(UAU UAA AAU AUU AUU AUU)	nt
51.	**ON23** 5′-α-D-ara(**TTA TTA TTA TAA AAT TAT**) **ON5** 5′-d(AAT AAT AAT ATT TTA ATA)	17.5
52.	**ON23** 5′-α-D-ara(**TTA TTA TTA TAA AAT TAT**) **ON7** 5′-r(AAU AAU AAU AUU UUA AUA)	18.0
53.	**ON6** 5′-d(TAT TAA AAT ATT ATT ATT) **ON24** 5′-α-D-ara (**ATA ATT TTA TAA TAA TAA**)	17.0
54.	**ON8** 5′-r(UAU UAA AAU AUU AUU AUU) **ON24** 5′-α-D-ara(**AAT AAT AAT ATT TTA ATA**)	16.5

[a] See captions below Table 1 and Table 2.

Table 6 α-L-ara *vs* α-D-ara hybridization in anti-parallel orientation[a]

Entry	Duplexes	T_m(°C)
55.	**ON9** 5′-d(TTA TTA TT α-L-ara(**A TA**)A AAT TAT) **ON15** 3′-d(AAT AAT AAα-D-ara(**T AT**)T TTA ATA)	25.0
56.	**ON11** 5′-d(TTA TTA TTα-D-ara(**A TA**)A AAT TAT) **ON13** 3′-d(AAT AAT AAα-L-ara(**T AT**)T TTA ATA)	27.0
57.	**ON10** 5′-d(TTA TTA α-L-ara(**TTA TAA**) AAT TAT) **ON16** 3′-d(AAT AAT α-D-ara(**AAT ATT**) TTA ATA)	17.0
58.	**ON12** 5′-d(TTA TTA α-D-ara(**TTA TAA**) AAT TAT) **ON14** 3′-d(AAT AAT α-L-ara(**AAT ATT**) TTA ATA)	16.0
59.	**ON21** 5′-α-L-ara(**TTA TTA TTA TAA AAT TAT**) **ON24** 3′-α-D-ara(**AAT AAT AAT ATT TTA ATA**)	nt
60.	**ON23** 5′-α-D-ara(**TTA TTA TTA TAA AAT TAT**) **ON22** 3′-α-L-ara(**AAT AAT AAT ATT TTA ATA**)	nt

[a] See captions below Table 1 and Table 2.

formation. As an expansion we also studied segments of three, six and fully modified α-L-ara:α-D-ara base pairs (Table 6, entries 55–60). Interestingly, duplexes of three and six α-L-ara/α-D-ara in central segments (entries 55–58) showed similar thermal stabilities as the duplexes with stereoregular central segments (entries 17, 18, 42 and 43) demonstrating a remarkable stereochemical space available for nucleic acid duplex formation. In contrast, no hybridization between fully modified α-L-ara and fully modified α-D-ara ONs was observed (entries 59–60).

Conclusion

We have successfully synthesized α-L-ara and α-D-ara phosphoramidite monomers and prepared fully modified as well as partially modified oligonucleotides thereof. Reduced duplex thermal stabilities relative to unmodified reference duplexes were observed for oligonucleotides containing a segment of three and six modifications of ara-T or ara-A monomers, whereas more stable duplexes were obtained when the central segment included exclusively α-ara-configured nucleotides (α-L-ara:α-L-ara or α-D-ara:α-D-ara or α-L-ara:α-D-ara base pairs). Fully modified α-L-ara oligonucleotides, neither in antiparallel nor in a parallel orientation were able to form duplexes towards DNA or RNA complement. We have confirmed reports[21] that fully modified α-D-ara oligonucleotides are able to form rather stable parallel duplexes with complementary α-D-ara oligonucleotides (also shown herein in the enantiomeric α-L-ara series). We have shown that fully modified α-L-ara oligonucleotides are unable to form parallel or antiparallel duplexes with complementary DNA/RNA, and confirmed parallel duplex formation of fully modified α-D-ara oligonucleotides with a DNA complement. Furthermore, we have for the first time shown parallel duplex formation between fully modified α-D-ara oligonucleotides and RNA complements. In summary, our results contribute to expanding the remarkable stereochemical space available for nucleic acid duplex formation which underlines the opportunity that prebiotic nucleic acid chemistry could have involved stereoirregular nucleic acid components.

Experimental

General

Reactions under anhydrous conditions were carried out under an atmosphere of nitrogen. After drying an organic phase over Na_2SO_4, filtration was performed. Solvents were of HPLC grade, of which DMF, pyridine, acetonitrile and dichloromethane were dried over molecular sieves (4 Å). TLC was run on Merck silica 60 F_{254} aluminium sheets. ^{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 (δH: $CDCl_3$ 7.26 ppm, DMSO-d_6 2.50; δC: $CDCl_3$ 77.0 ppm, DMSO-d_6 39.4). Chemical shifts are reported in ppm relative to either tetramethylsilane or the deuterated solvent as a 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 NMR experiments (the assignments of methylene protons/methylene carbons may be interchanged). Coupling constants (J values) are given in Hz. MALDI-HRMS were recorded in positive ion mode on an Ion Spec Fourier transform mass spectrometer. The ^{1}H and ^{13}C NMR data of the α-D-arabino-nucleosides were identical with the corresponding enantiomeric α-L-arabino-nucleosides, and as these nucleosides were synthesized by the same synthetic route in similar scales (but starting from enantiomeric starting materials), we report only yield, optical rotation and high resolution mass spectral data for the α-D-arabino-nucleosides; only if the experimental procedure has been changed relative to the literature procedure have synthetic details been added. All the α-D-arabino-nucleoside triols **1D-4D** were synthesized from D-arabinose and their structural assignments were confirmed by comparison of the NMR data with those reported in the literature.[21,22]

9-(α-L-Arabinofuranosyl)-6-*N*-benzoyladenine (5). The nucleoside **2** (2.00 g, 7.49 mmol) was co-evaporated with pyridine (2 × 50 mL) and redissolved in pyridine (50 mL). Trimethylsilyl chloride (TMSCl) (3.70 mL, 29.24 mmol) was added and the mixture was stirred at RT for 1 h whereupon benzoyl chloride (1.16 mL, 8.98 mmol) was added dropwise. The resulting mixture was stirred for 4 h at RT whereupon water (10 mL) was added. After stirring for 5 min at RT, aqueous ammonia (18 mL, ~29% v/v) was added and the resulting mixture was stirred for 15 min at RT and

then evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel (3–4% methanol in ethyl acetate, v/v) to give nucleoside **5** as a white solid material (2.30 g, 76%). R_f = 0.2 (10% MeOH in CH_2Cl_2, v/v). $[\alpha]_D^{32}$ = –3.8 (*c* 0.1, MeOH). ^{1}H NMR (DMSO-d_6) δ 3.40–3.69 (2H, m, H-5′), 4.13 (1H, q, *J* = 5.4 Hz, H-3′), 4.24–4.29 (1H, m, H-4′), 4.75 (1H, q, *J* = 5.4 Hz, H-2′), 4.96 (1H, t, *J* = 5.7 Hz, OH-5′), 5.69 (1H, d, *J* = 4.8 Hz, OH-3′), 5.88 (1H, d, *J* = 5.1 Hz, OH-2′), 6.02 (1H, d, *J* = 5.1 Hz, H-1′), 7.52–7.69 (3H, m, H-3″, H-4″ and H-5″), 8.04–8.07 (2H, m, H-2″ and H-6″), 8.70 (1H, s, H-2), 8.78 (1H, s, H-8) and 11.2 (1H, br s, NH). ^{13}C NMR (DMSO-d_6) δ 61.1 (C-5′), 75.9 (C-3′), 79.2 (C-2′), 85.6 (C-4′), 88.6 (C-1′), 125.9 (C-5), 128.5, 132.5, 133.4 (Ar-Bz), 143.6 (C-8), 150.3 (C-4), 151.6 (C-2), 152.1 (C-6) and 165.6 (*C*OPh). MALDI-HRMS *m/z* 394.1124 ([M + Na]$^+$, calculated for $C_{17}H_{17}N_5O_5Na$ 394.1121).

1-(α-L-Arabinofuranosyl)-4-*N*-benzoylcytosine (6)[32]. The nucleoside **6** was obtained as a white solid material (92% yield). MALDI-HRMS *m/z* 370.1043 ([M + Na]$^+$, calculated for $C_{16}H_{17}N_3O_6Na$ 370.1009). NMR data were identical with the reported data.[32]

9-(α-L-Arabinofuranosyl)-2-*N*-isobutyrylguanine (7). The nucleoside **4** (3.20 g, 11.30 mmol) was dissolved in anhydrous pyridine (100 mL) and TMSCl (7.17 mL, 56.53 mmol) was added. After stirring for 30 min at RT, isobutyric anhydride (2.25 mL, 13.56 mmol) was added and the resulting mixture was stirred for 2 h at RT. H_2O (16 mL) was added and after stirring for 5 min at RT, aqueous ammonia (32 mL, ~29%) was added and the resulting mixture was stirred at RT for another 15 min whereupon it was evaporated to dryness under reduced pressure. The resulting residue was purified by silica gel column chromatography (8–11% methanol in chloroform, v/v) to afford nucleoside **7** as a white solid material (3.12 g, 78%). R_f = 0.51 (15% MeOH in CH_2Cl_2, v/v). $[\alpha]_D^{32}$ = –16.5 (*c* 0.1, MeOH). ^{1}H NMR (DMSO-d_6) δ 1.11 (6H, d, *J* = 6.7 Hz, CH(C*H*$_3$)$_2$), 2.50 (1H, sept, *J* = 6.7 Hz, C*H*(CH$_3$)$_2$), 3.48 (1H, dd, *J* = 11.7 and 4.8 Hz, H-5′$_\alpha$), 3.60 (1H, dd, *J* = 11.7 and 3.2 Hz, H-5′$_\beta$), 4.01 (1H, t, *J* = 5.6 Hz, H-3′), 4.15 (1H, q, *J* = 3.9 Hz, H-4′), 4.53 (1H, t, *J* = 5.0 Hz, H-2′), 5.76 (1H, d, *J* = 4.8 Hz, H-1′), 7.11–7.45 (3H, 3 s, OH-5′, OH-3′ and OH -2′), 8.21 (1H, s, H-8), 11.69 (1H, br s, NH), 12.09 (1H, br s, N*H*COCH(CH$_3$)$_2$). ^{13}C NMR (DMSO-d_6) δ 19.5 (CH(*C*H$_3$)$_2$), 35.4 (*C*H(CH$_3$)$_2$), 61.8 (C-5′), 75.9 (C-3′), 80.3 (C-2′), 86.0 (C-4′), 88.7 (C-1′), 120.9 (C-5), 139.0 (C-8), 148.7 (C-4), 149.3 (C-2), 155.6 (C-6), 180.8 (*C*OCH(CH$_3$)$_2$). MALDI-HRMS *m/z* 376.1257 ([M + Na]$^+$, calculated for $C_{14}H_{19}N_5O_6Na$ 376.1227).

1-(3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-α-L-arabinofuranosyl)thymine (8)[33]. The nucleoside **8** was obtained as a white amorphous solid material (80% yield). $[\alpha]_D^{32}$ = –12.6 (*c* 0.1, MeOH). MALDI-HRMS *m/z* 523.2255 ([M + Na]$^+$, calculated for $C_{22}H_{40}N_2O_7Si_2Na$ 523.2266). NMR data were identical with the reported data.[33]

9-(3′,5′-*O*-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (9). The nucleoside **5** (2.30 g, 6.19 mmol) was co-evaporated with pyridine and dissolved in anhydrous pyridine (100 mL). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (2.13 mL, 6.81 mmol) was added dropwise under stirring at RT and the mixture was stirred for 5 h at RT under an atmosphere of nitrogen. The solvent was removed under reduced pressure and the residue diluted with dichloromethane (200 mL). The organic phase was washed successively with saturated aqueous solutions of $NaHCO_3$ (2 × 200 mL) and brine (2 × 200 mL) and was dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel (80% ethyl acetate in petroleum ether, v/v) to afford nucleoside **9** as a white solid material (3.06 g, 81%). R_f = 0.28 (75% EtOAc in petroleum ether, v/v). $[\alpha]_D^{32}$ = –22.5 (*c* 0.1, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.05–1.11 (28H, m, 4 × C*H*(C*H*$_3$)$_2$), 3.94 (2H, br s, H-5′), 4.39 (2H, br s, H-3′ and H-4′), 5.14 (1H, br s, H-2′), 5.99 (2H, d, *J* = 6.0 Hz, H-1′ and OH-2′), 7.52–7.63 (3H, m, H-3″, H-4″ and H-5″), 8.07 (2H, d, *J* = 7.3 Hz, H-2″ and H-6″), 8.78 (2H, s, H-2 and H-8) 11.19 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 12.0, 12.2, 12.5, 12,9 (4 × *C*H(CH$_3$)$_2$), 16.8, 16.90, 17.1, 17.2, (4 × CH(*C*H$_3$)$_2$), 60.9 (C-5′), 75.0 (C-3′), 76.8 (C-2′), 81.3 (C-4′), 87.0 (C-1′), 126.0 (C-5), 128.3 (C-3″ and C-5″), 128.4 (C-2″ and C-6″), 132.2 (C-4″), 133.3 (C-1″), 143.9 (C-8), 150.4 (C-6), 151.6 (C-4), 152.2 (C-2), 165.5 (*C*OPh). MALDI-HRMS *m/z* 636.2624 ([M + Na]$^+$, calculated for $C_{29}H_{43}N_5O_6Si_2Na$ 636.2644).

1-(3′,5′-*O*-(1,1,3,3-Tetraisopropylidisiloxane-1,3-diyl)-α-L-arabinofuranosyl)-4-*N*-benzoylcytosine (10). The nucleoside **6** (2.50 g, 7.20 mmol) was co-evaporated with pyridine and dissolved in anhydrous pyridine (100 mL) under stirring. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (2.48 mL, 7.92 mmol) was added and the resulting mixture was stirred for 3 h at RT under an atmosphere of nitrogen. The solvent was removed under reduced pressure and the residue diluted with dichloromethane (200 mL). The organic phase was washed successively with saturated aqueous solutions of $NaHCO_3$ (2 × 200 mL) and brine (2 × 200 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel (80% ethyl acetate in petroleum ether, v/v) to afford nucleoside **10** as a white solid material (2.77 g, 66%). R_f = 0.6 (30% EtOAc in petroleum ether, v/v). $[\alpha]_D^{32}$ = +14.5 (*c* 0.1, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.00–1.12 (28H, m, 4 × C*H*(C*H*$_3$)$_2$), 3.91 (1H, m, H-4′), 4.03 (1H, dd, *J* = 13.0 and 2.5 Hz, H-5′$_\alpha$), 4.13 (1H, dd, *J* = 13.0 and 3.2 Hz, H-5′$_\beta$), 4.24 (1H, m, H-3′), 4.47 (1H, t, *J* = 7.6 Hz, H-2′), 4.64 (1H, s, OH-2′), 5.58 (1H, d, *J* = 4.3 Hz, H-1′), 7.52 (2H, t, *J* = 7.1 Hz, H-3″ and H-5″), 7.60–7.67 (2H, m, H-5 and H-4″), 7.89 (1H, d, *J* = 7.2 Hz, H-2″ and H-6″), 8.03 (1H, d, *J* = 7.2 Hz, H-6), 8.70 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 12.8, 13.2, 13.4, 13,8 (4 × *C*H(CH$_3$)$_2$), 17.2, 17.4, 17.6, 17.8 (4 × CH(*C*H$_3$)$_2$), 61.1 (C-5′), 75.8 (C-3′), 82.8 (C-2′), 83.1 (C-4′), 94.2 (C-1′), 96.8 (C-5), 127.8 (C-3″ and C-5″), 129.5 (C-2″ and C-6″), 133.7 (C-6 and C-4″), 143.0 (C-2), 162.9 (C-4), 166.1 (*C*OPh). MALDI-HRMS *m/z* 612.2507 ([M + Na]$^+$, calculated for $C_{28}H_{43}N_3O_7Si_2Na$ 612.2531).

9-(3′,5′-*O*-(1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl)-α-L-arabinofuranosyl)-2-*N*-isobutyrylguanine (11). The nucleoside **7** (2.90 g, 8.21 mmol) was co-evaporated with pyridine (2 × 20 mL) and dissolved in anhydrous pyridine (100 mL) under stirring. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (2.83 mL, 9.03 mmol) was added dropwise and the resulting mixture was stirred for 12 h at RT under an atmosphere of nitrogen. The solvent was removed under reduced pressure and the residue diluted with dichloromethane (120 mL). The organic phase was washed successively with saturated aqueous solutions of $NaHCO_3$ (2 × 120 mL) and brine (2 × 120 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was

purified by column chromatography over silica gel (35% ethyl acetate in petroleum ether, v/v) to afford nucleoside **11** as a white solid material (3.36 g, 76%). $R_f = 0.45$ (30% MeOH in CH_2Cl_2, v/v). $[\alpha]_D^{32} = +14.0$ (*c* 0.1, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.04–1.22 (28H, m, 4 × C*H*(CH_3)$_2$), 1.28 (6H, d, *J* = 6.6 Hz, COCH(CH_3)$_2$), 2.78 (1H, sept, *J* = 6.6 Hz, COC*H*(CH$_3$)$_2$), 3.96 (2H, br s, H-5′), 4.21 (1H, br s, H-4′), 4.45 (1H, t, *J* = 8.2 Hz, H-3′), 5.13 (1H, br s, H-2′), 5.78 (1H, d, *J* = 5.3 Hz, H-1′), 6.30 (1H, s, OH-2′), 7.73 (1H, s, H-8), 10.36 (1H, s, NH), 12.0 (1H, br s, N*H*COCH(CH$_3$)$_2$). ^{13}C NMR ($CDCl_3$) δ 12.7, 13.1, 13.4, 13.8 [4 × *C*H(CH$_3$)$_2$], 17.2, 17.3, 17.3, 17.4, 17.6, 17.6, 17.8 (COCH(*C*H$_3$)$_2$ and 4 × CH(*C*H$_3$)$_2$), 36.6 (CO*C*H(CH$_3$)$_2$), 61.5 (C-5′), 74.9 (C-3′), 78.9 (C-2′), 82.8 (C-4′), 89.8 (C-1′), 120.6 (C-5), 138.8 (C-8), 148.1 (C-4), 148.5 (C-2), 155.6 (C-6), 179.9 [*C*OCH(CH$_3$)$_2$]. MALDI-HRMS *m/z* 596.2993 ([M + H]$^+$, calculated for $C_{26}H_{46}N_5O_7Si_2$ 596.2930).

1-(2′-*O*-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-L-arabinofuranosyl)thymine (12). The nucleoside **8** (3.35 g, 6.69 mmol) was co-evaporated with pyridine and dissolved in anhydrous pyridine (65 ml) under stirring. Acetic anhydride (0.94 ml, 9.91 mmol) was added and the resulting mixture was stirred at RT under an atmosphere of nitrogen for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane and was washed successively with saturated aqueous solutions of $NaHCO_3$ (2 × 180 ml) and brine (2 × 180 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel (20–25% ethyl acetate in petroleum ether, v/v) to afford nucleoside **12** as a white solid material (3.11 g, 86%). R_f = 0.7 (5% MeOH in CH_2Cl_2, v/v). $[\alpha]_D^{32} = -8.9$ (*c* 0.1, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.02–1.11 (28H, m, 4 × C*H*(CH_3)$_2$), 1.95 (3H, s, CH_3–5), 2.09 (3H, s, COCH_3), 3.97 (2H, d, *J* = 4.2 Hz, H-5′), 4.21 (1H, m, H-4′), 4. 56 (1H, t, *J* = 7.8 Hz, H-3′), 5.55 (1H, t, *J* = 6.8 Hz, H-2′), 5.87 (1H, d, *J* = 5.7 Hz, H-1′), 7.15 (1H, s, H-6) and 9.01 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ 12.8, 12.9, 13.2, 13.5, 13.7 (4 × *C*H(CH$_3$)$_2$), 17.1, 17.2, 17.29, 17.6, 17.7 (4 × CH(*C*H$_3$)$_2$), 20.9 (CO*C*H$_3$), 62.2 (C-5′), 74.2 (C-3′), 80.1 (C-2′), 83.5 (C-4′), 87.5 (C-1′), 111.9 (C-6), 136.0 (C-5), 150.9 (C-2), 163.9 (C-4) and 170.5 (*C*OCH$_3$). MALDI-HRMS *m/z* 565.2372 ([M + Na]$^+$, calculated for $C_{24}H_{42}N_2O_8Si_2Na$ 565.2371).

9-(2′-*O*-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (13). The nucleoside **9** (3.00 g, 4.89 mmol) was co-evaporated with pyridine and dissolved in anhydrous pyridine (30 ml) under stirring. Acetic anhydride (0.74 ml, 7.83 mmol) was added and the resulting mixture was stirred at RT under an atmosphere of nitrogen for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane and was washed successively with saturated aqueous solutions of $NaHCO_3$ (2 × 150 ml) and brine (2 × 150 ml) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel (45–60% ethyl acetate in petroleum ether, v/v) to give nucleoside **13** as a white solid material (2.87 g, 90%). R_f = 0.44 (75% EtOAc in petroleum ether, v/v). $[\alpha]_D^{32} = -14.6$ (*c* 0.1, MeOH). ^{1}H NMR ($CDCl_3$) δ 0.91–1.14 (28H, m, 4 × C*H*(CH_3)$_2$), 2.08 (3H, s, CO*C*H$_3$), 4.0–4.05 (2H, m, H-5′), 4.53 (1H, q, *J* = 3.5 Hz, H-4′), 4.67 (1H, t, *J*= 8.0 Hz, H-3′), 6.07–6.13 (2H, m, H-1′ and H-2′), 7.46–7.57 (3H, m, H-3″, H-4″ and H-5″), 8.03 (2H, d, *J* = 7.3 Hz, H-2″ and H-6″), 8.19 (1H, s, H-2), 8.80 (1H, s, H-8), 9.41 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 12.8, 12.9, 13.2, 13.4, 134, 13.8 (4 × -*C*H(CH$_3$)$_2$), 17.2, 17.2, 17.3, 17.4, 17.6, 17.6, 17.7 (4 × -CH(*C*H$_3$)$_2$), 20.8 (CO*C*H$_3$), 61.7 (C-5′), 74.9 (C-3′), 80.9 (C-2′), 83.7 (C-4′), 86.7 (C-1′), 123.6 (C-5), 128.3 (C-3″ and C-5″), 128.85 (C-2″ and C-6″), 133.0 (C-4″), 134.1 (C-1″), 142.1 (C-8), 150.1 (C-6), 152.1 (C-4), 153.2 (C-2), 165.7 (*C*OPh), 170.3 (*C*OCH$_3$). MALDI-HRMS *m/z* 678.2724 ([M + Na]$^+$, calculated for $C_{31}H_{45}N_5O_7Si_2Na$ 678.2749).

1-(2′-*O*-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-L-arabinofuranosyl)-4-*N*-benzoylcytosine (14). The nucleoside **10** (2.60 g, 4.42 mmol) was co-evaporated with pyridine and dissolved in anhydrous pyridine (30 mL) under stirring. Acetic anhydride (0.67 mL, 7.07 mmol) was added and the resulting mixture was stirred at RT under an atmosphere of nitrogen for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane and was washed successively with saturated aqueous solutions of $NaHCO_3$ (2 × 50 mL) and brine (2 × 50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel (60% ethyl acetate in petroleum ether, v/v) to afford nucleoside **14** as a white solid material (2.45 g, 88%). R_f = 0.51 (30% EtOAc in petroleum ether, v/v). $[\alpha]_D^{32} = -22.6$ (*c* 0.1, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.01–1.12 (28H, m, 4 × C*H*(CH_3)$_2$), 2.11 (3H, s, CO*C*H$_3$), 3.97–4.03 (2H, m, H-5′), 4.29–4.34 (1H, m, H-4′), 4.59 (1H, t, *J* = 7.4 Hz, H-3′), 5.61 (1H, t, *J* = 5.2 Hz, H-2′), 5.98 (1H, d, *J* = 5.2 Hz, H-1′), 7.48–7.53 (3H, m, H-5, H-3″ and H-5″), 7.61 (1H, m, H-4″), 7.79 (2H, d, *J* = 7.4 Hz, H-2″ and H-6″), 7.89 (1H, d, *J* = 7.3 Hz, H-6), 8.70 (1H, s, NH). ^{13}C NMR ($CDCl_3$) δ 14.5, 15.0, 15.3, 15.5 (4 × *C*H(CH$_3$)$_2$), 18.9, 19.0, 19.3, 19.4, 19.5 (4 × CH(*C*H$_3$)$_2$), 22.7 ((CO*C*H$_3$), 63.9 (C-5′), 76.2 (C-3′), 82.5 (C-2′), 86.0 (C-4′), 91.5 (C-1′), 99.4 (C-5), 129.9 (C-2″ and C-6″), 131.0 (C-3″ and C-5″), 135.1 (C-4″ and C-6), 146.5 (C-2 & C-1″), 148.2 (C-4), 164.9 (*C*OPh), 172.2 (*C*OCH$_3$). MALDI-HRMS *m/z* 654.2629 ([M + Na]$^+$, calculated for $C_{30}H_{45}N_3O_8Si_2Na$ 654.2637).

9-[2′-*O*-Acetyl-3′,5′-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-α-L-arabinofuranosyl)-2-*N*-isobutyrylguanine (15). The nucleoside **11** (3.20 g, 5.93 mmol) was co-evaporated with pyridine and dissolved in anhydrous pyridine (60 mL) under stirring. Acetic anhydride (0.9 mL, 9.49 mmol) was added and the resulting mixture was stirred at RT under an atmosphere of nitrogen for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (50 mL) and was washed with saturated aqueous solutions of $NaHCO_3$ (2 × 60 mL) and brine (2 × 60 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by column chromatography over silica gel (40% ethyl acetate in petroleum ether, v/v) to afford nucleoside **15** as a white solid material (2.75 g, 80%). R_f = 0.35 (30% EtOAc in petroleum ether, v/v). $[\alpha]_D^{32} = -38.9$ (*c* 0.1, MeOH). ^{1}H NMR ($CDCl_3$) δ 1.03–1.12 (28H, m, 4 × C*H*(CH_3)$_2$), 1.24 (6H, d, *J* = 5.7 Hz COCH(CH_3)$_2$), 2.10 (3H, s, COCH_3), 2.69 (1H, sept, J = 5.7 Hz, COC*H*(CH$_3$)$_2$), 3.91–4.05 (2H, m, H-5′), 4.31 (1H, br s, H-4′), 4.58 (1H, t, *J* = 6.9 Hz, H-3′), 5.85 (1H, d, *J* = 3.2 Hz, H-2′), 6.05 (1H, br s, H-1′), 7.93 (1H, s, H-8), 9.20 (1H, br s, NH), 12.09 (1H, br s, N*H*COCH(CH$_3$)$_2$). ^{13}C NMR ($CDCl_3$) δ 12.7, 13.2, 13.5, 13.7 (4 × *C*H(CH$_3$)$_2$), 17.2, 17.3, 17.5, 17.6, 17.6, 17.7, 19.3 (4 × CH(*C*H$_3$)$_2$ and COCH(*C*H$_3$)$_2$), 20.9 (CO*C*H$_3$), 36.7 (CO*C*H(CH$_3$)$_2$), 62.1

(C-5′), 75.8 (C-3′), 80.9 (C-2′), 83.9 (C-4′), 86.5 (C-1′), 122.0 (C-5), 138.3 (C-8), 148.1 (C-4), 148.6 (C-2), 155.9 (C-6), 170.3 (*C*OCH$_3$) 179.0 (*C*OCH(CH$_3$)$_2$). MALDI-HRMS *m/z* 638.3109 ([M + H]$^+$, calculated for $C_{28}H_{48}N_5O_8Si_2$ 638.3035).

1-(2′-*O*-Acetyl-α-L-arabinofuranosyl)thymine (16). The nucleoside **12** (2.40 g, 4.42 mmol) was co-evaporated with anhydrous acetonitrile (2 × 50 mL) and dissolved in anhydrous acetonitrile (80 mL). To this solution was added Et$_3$N·3HF dropwise (1.62 mL, 9.95 mmol) dissolved in acetonitrile (3.0 mL). The mixture was stirred at RT under an atmosphere of nitrogen for 5 h whereupon the solvent was evaporated under reduced pressure. The residue was co-evaporated with toluene (2 × 50 mL) and purified by column chromatography over silica gel (2–5% methanol in chloroform, v/v) to afford the nucleoside **16** as a white solid material (1.21 g, 91%). R_f = 0.2 (10% MeOH in CH_2Cl_2, v/v). $[\alpha]_D^{32} = -7.0$ (*c* 0.1, MeOH). ^{1}H NMR (DMSO-d_6) δ 1.79 (3H, s, C-5CH$_3$), 2.04 (3H, s, COC*H*$_3$), 3.46 (1H, d, *J* = 11.6 Hz, H-5′$_\alpha$), 3.56 (1H, d, *J* = 11.6 Hz, H-5′$_\beta$), 4.20 (2H, br s, H-3′ and H-4′), 4.91 (1H, br s, OH-5′), 5.25 (1H, t, *J* = 4.9 Hz, H-2′), 5.73 (2H, d, *J* = 4.1 Hz, H-1′), 5.86 (1H, br s, OH-3′), 7.64 (1H, s, H-6) and 11.25 (1H, br s, NH). ^{13}C NMR (DMSO-d_6) δ 12.1 (C-5CH$_3$), 20.5 (CO*C*H$_3$), 62.1 (C-5′), 75.2 (C-3′), 83.6 (C-2′), 87.7 (C-4′), 89.2 (C-1′), 125.1 (C-5), 129.4 (C-3″ and C-5″), 129.7 (C-2″ and C-6″), 133.9 (C-4″), 134.9 (C-1″), 144.8 (C-8), 151.2 (C-6), 153.1 (C-4), 153.2 (C-2), 165.5 (*C*OPh) and 171.9 (*C*OCH$_3$). MALDI-HRMS *m/z* 323.0850 ([M + Na]$^+$, calculated for $C_{12}H_{16}N_2O_7Na$ 323.0849).

9-(2′-*O*-Acetyl-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (17). The nucleoside **13** (2.20 g, 3.35 mmol) was co-evaporated with anhydrous acetonitrile (2 × 50 mL) and dissolved in anhydrous acetonitrile (55 ml). To this solution was dropwise added Et$_3$N·3HF (1.23 mL, 7.55 mmol) dissolved in acetonitrile (3.0 mL). The mixture was stirred at RT under an atmosphere of nitrogen for 5 h whereupon the solvent was evaporated under reduced pressure. The residue was co-evaporated with toluene (2 × 50 mL) and purified by column chromatography over silica gel (70–80% ethyl acetate in petroleum ether, v/v) to afford nucleoside **17** as a white solid material (1.28 g, 93%). R_f = 0.35 (10% MeOH in CH_2Cl_2, v/v). $[\alpha]_D^{32} = -38.6$ (*c* 0.1, MeOH). ^{1}H NMR (DMSO-d_6) δ 2.06 (3H, s, CO*C*H$_3$), 3.60 (2H, m, H-5′), 4.29–4.35 (1H, m, H-4′), 4.37–4.39 (1H, m, H-3′), 4.94 (1H, t, *J* = 5.6 Hz, OH-5′), 5.85 (1H, t, *J* = 4.7 Hz, H-2′), 5.95 (1H, d, *J* = 4.9 Hz, OH-3′), 6.21 (1H, d, *J* = 4.2 Hz, H-1′), 7.53–7.67 (3H, m, H-3″, H-4″, H-5″), 8.05 (2H, d, *J* = 7.3 Hz, H-2″ and H-6″), 8.65 (1H, s, H-2), 8.78 (1H, s, H-8). ^{13}C NMR (DMSO-d_6) δ 19.0 (CO*C*H$_3$), 58.9 (C-5′), 71.6 (C-3′), 79.7 (C-2′), 83.9 (C-4′), 84.9 (C-1′), 124.2 (C-5), 126.9 (C-3″, C-5″, C-2″ and C-6″), 130.9 (C-4″), 131.8 (C-1″), 141.9 (C-8), 148.9 (C-6), 150.2 (C-4), 150.4 (C-2), 164.1 (*C*OPh), 168.4 (*C*OCH$_3$). MALDI-HRMS *m/z* 436.1213 ([M + Na]$^+$, calculated for $C_{19}H_{19}N_5O_6Na$ 436.1227).

1-(2′-*O*-Acetyl-α-L-arabinofuranosyl)-4-*N*-benzoylcytosine (18). The nucleoside **14** (2.30 g, 3.65 mmol) was co-evaporated with anhydrous acetonitrile (2 × 30 mL) and dissolved in anhydrous acetonitrile (60 mL). To this solution was dropwise added Et$_3$N·3HF (1.33 mL, 8.21 mmol) dissolved in acetonitrile (3.0 mL) and the resulting mixture was stirred at RT under an atmosphere of nitrogen for 5 h. The solvent was evaporated under reduced pressure and the residue was co-evaporated with toluene (2 × 30 mL) and purified by column chromatography over silica gel (70–80% ethyl acetate in petroleum ether, v/v) to afford nucleoside **18** as a white solid material (1.13 g, 80%). R_f = 0.52 (10% MeOH in CH_2Cl_2, v/v). $[\alpha]_D^{32} = -31.6$ (*c* 0.1, MeOH). ^{1}H NMR (DMSO-d_6) δ 2.08 (3H, s, CO*C*H$_3$), 3.54–3.56 (2H, m, H-5′), 4.16 (1H, q, *J* = 3.6 Hz, H-4′), 4.38 (1H, t, *J* = 4.3 Hz, H-3′), 4.96 (1H, t, *J* = 5.6 Hz, OH-5′), 5.29 (1H, br s, H-2′), 5.74 (1H, d, *J* = 4.1 Hz, OH-3′), 5.85 (1H, d, *J* = 2.1 Hz, H-1′), 7.36 (1H, d, *J* = 5.8 Hz, H-5), 7.51 (2H, t, *J* = 7.3 Hz, H-3″ and H-5″), 7.62 (1H, t, *J* = 7.1 Hz, H-4″), 8.01 (2H, d, *J* = 7.4 Hz, H-2″ and H-6″), 8.15 (1H, d, *J* = 7.2 Hz, H-6), 11.25 (1H, s, NH). ^{13}C NMR (DMSO-d_6) δ 20.6 (CO*C*H$_3$), 60.8 (C-5′), 73.0 (C-3′), 81.3 (C-2′), 88.2 (C-4′), 90.8 (C-1′), 95.9 (C-5), 128.1 (C-2″ and C-6″), 128.4 (C-3″ and C-5″), 132.7 (C-4″), 133.1 (C-1″), 146.1 (C-6), 154.4 (C-4), 163.3 (C-2), 167.3 (*C*OPh), 169.5 (*C*OCH$_3$). MALDI-HRMS *m/z* 412.1120 ([M + Na]$^+$, calculated for $C_{18}H_{19}N_3O_7Na$ 412.1115).

9-(2′-*O*-Acetyl-α-L-arabinofuranosyl)-2-*N*-isobutyrylguanine (19). The nucleoside **15** (2.60 g, 4.47 mmol) was co-evaporated with anhydrous acetonitrile (2 × 30 mL) and dissolved in anhydrous acetonitrile (60 mL). To this solution was dropwise added Et$_3$N·3HF (1.64 mL, 10.06 mmol) dissolved in acetonitrile (3.0 mL) and the resulting mixture was stirred at RT under an atmosphere of nitrogen for 5 h. The solvent was evaporated under reduced pressure and the residue was coevaporated with toluene (2 × 50 mL) and purified with column chromatography (4–5% methanol in chloroform, v/v) over silica gel to afford nucleoside **19** as a white solid material (1.14 g, 79%). R_f = 0.35 (70% EtOAc in petroleum ether, v/v). $[\alpha]_D^{32} = -27.0$ (*c* 0.1, MeOH). ^{1}H NMR (DMSO-d_6) δ 1.12 (6H, d, *J* = 6.6 Hz, COCH(C*H*$_3$)$_2$), 2.06 (3H, s, COCH$_3$), 2.78 (1H, sept, *J* = 6.6 Hz, COC*H*(CH$_3$)$_2$), 3.52–3.63 (2H, m, H-5′), 4.21–4.24 (2H, m, H-3′ and H-4′), 4.94 (1H, t, *J* = 5.2 Hz, OH-5′), 5.58 (1H, t, *J* = 4.5 Hz, H-2′), 5.89 (1H, d, *J* = 4.8 Hz, OH-3′), 5.95 (1H, d, *J* = 3.8 Hz, H-1′), 8.22 (1H, s, H-8), 11.59 (1H, br s, NH), 12.12 (1H, br s, N*H*COCH(CH$_3$)$_2$). ^{13}C NMR (DMSO-d_6) δ 18.8 (COCH(*C*H$_3$)$_2$), 20.5 (CO*C*H$_3$), 34.7 (CO*C*H(CH$_3$)$_2$), 60.4 (C-5′), 73.0 (C-3′), 81.5 (C-2′), 85.3 (C-4′), 85.6 (C-1′), 120.1 (C-5), 138.1 (C-8), 148.1 (C-4), 148.4 (C-2), 154.8 (C-6), 169.8 (*C*OCH$_3$) 180.1 [*C*OCH(CH$_3$)$_2$]. MALDI-HRMS *m/z* 418.1313 ([M + Na]$^+$, calculated for $C_{16}H_{21}N_5O_7Na$ 418.1333).

1-(2′-*O*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)thymine (20). The nucleoside **16** (0.60 g, 1.99 mmol) was coevaporated with anhydrous pyridine (2 × 5 mL) and then dissolved in a mixture of dichloromethane and pyridine (30 mL, 4:1). 4,4′-Dimethoxytrityl chloride (DMTCl; 0.74 g, 2.19 mmol) was added under an atmosphere of nitrogen and the resulting mixture was stirred at RT for 10 h. Additional DMTCl (0.075 g, 0.22 mmol) was added after 10 h and reaction mixture was stirred for another 2 h. The reaction mixture was evaporated to dryness and the residue was dissolved in dichloromethane (60 mL) whereupon washing was performed using a saturated aqueous solution of NaHCO$_3$ (2 × 20 mL). The organic phase was dried over Na_2SO_4, filtered and evaporated to dryness under reduced pressure to get a residue which was purified by column chromatography over silica (60–70% ethyl acetate in petroleum ether, v/v) to afford nucleoside **20** (1.01 g, 84%) as a white solid material. R_f = 0.4 (5% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (DMSO-d_6) δ 1.81 (3H, d,

$J = 0.9$ Hz, CH_3-5), 1.99 (3H, s, $COCH_3$), 3.12 (2H, d, $J = 4.5$ Hz, H-5′), 3.72 (6H, s, $2 \times OCH_3$), 4.22 (1H, q, $J = 5.1$ Hz, H-3′), 4.46 (1H, q, $J = 4.5$ Hz, H-4′), 5.24 (1H, t, $J = 4.5$ Hz, H-2′), 5.84–5.88 (2H, m, H-1′ and 3′-OH), 6.91 (4H, d, $J = 8.4$ Hz, ArH), 7.23–7.42 (9H, m, ArH), 7.67 (1H, d, $J = 0.9$ Hz, H-6) and 11.35 (1H, s, NH). ^{13}C NMR (DMSO-d_6) δ 12.1 (C-5CH_3), 20.4 (COCH_3), 55.0 ($2 \times OCH_3$), 62.8 (C-5′), 73.2 (C-3′), 80.7 (C-2′), 83.9 (C-4′), 85.4 (C-1′), 87.8 (C(Ph)$_3$), 109.3 (C-5), 113.2, 126.7, 127.6, 127.8, 129.6, 135.4, 135.4, 136.9, 144.7, 150.4, 158.0 (Ar-DMT, C-6 and C-2), 163.8 (C-4), 169.6 (COCH_3).MALDI-HRMS m/z 625.2146 ([M + Na]$^+$, calculated for $C_{33}H_{34}N_2O_9Na$ 625.2156).

9-(2′-*O*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (21). The nucleoside **17** (0.65 g, 1.57 mmol) was co-evaporated with pyridine (2×15 mL) and then dissolved in a mixture of dichloromethane and pyridine (50 mL, 4:1). DMTCl (0.586 g, 1.72 mmol) was added and the resulting mixture was stirred for 12 h under an atmosphere of nitrogen. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in dichloromethane (100 ml) whereupon washing was performed using a saturated aqueous solution of $NaHCO_3$ (2×30 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel using (75–80% ethyl acetate in petroleum ether, containing 0.5% Et_3N, v/v/v) to afford nucleoside **21** as a white solid material (0.87 g, 78%). $R_f = 0.36$ (5% MeOH in CH_2Cl_2, v/v). ^{1}H NMR ($CDCl_3$) δ 2.06 (3H, s, $COCH_3$), 3.34 (2H, m, H-5′), 3.78 (6H, s, $2 \times OCH_3$), 4.49–4.53 (1H, m, H-4′), 4.57 (1H, q, $J = 4.8$ Hz, H-3′), 5.52 (1H, s, H-2′), 5.99 (1H, d, $J = 1.5$ Hz, H-1′), 6.10 (1H, d, $J = 9.6$ Hz, OH-3′), 6.88 (4H, d, $J = 8.7$ Hz, Ar-H), 7.18–7.62 (12H, m, Ar-H), 8.01 (2H, d, $J = 7.2$ Hz, Ar-H), 8.14 (1H, s, C-2H), 8.77 (1H, s, C-8H) and 9.21 (1H, br s, NHCOPh). ^{13}C NMR ($CDCl_3$) δ 20.7 (COCH_3) 55.3 ($2 \times OCH_3$), 62.8 (C-5′), 77.0 (C-3′), 85.1 (C-2′), 86.3 (C(Ph)$_3$), 87.9 (C-4′), 90.2 (C-1′), 113.2 (Ar-DMT), 123.7 (C-5), 126.9, 127.9, 128.0, 128.2, 129.0, 130.1, 133.1, 133.5, 135.8, 135.9 (Ar-Bz, Ar-DMT), 143.2 (C-8), 144.7 (Ar-DMT), 150.2 (C-6), 150.5 (C-4), 152.3 (C-2), 158.6 (OCH_3), 164.6 (COPh), 170.6 (COCH_3). MALDI-HRMS m/z 738.2582 ([M + Na]$^+$, calculated for $C_{40}H_{37}N_5O_8Na$ 738.2534).

9-(2′-*O*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)-4-*N*-benzoylcytosine (22). The nucleoside **18** (0.50 g, 1.29 mmol) was co-evaporated with pyridine (2×10 mL) and then dissolved in a mixture of dichloromethane and pyridine (30 mL, 4:1). DMTCl (0.48 g, 1.41 mmol) was added and the resulting mixture was stirred for 12 h under an atmosphere of nitrogen. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in dichloromethane (60 mL) whereupon washing was performed using a saturated aqueous solution of $NaHCO_3$ (2×20 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel (75–80% ethyl acetate in petroleum ether, v/v) to give nucleoside **22** as a white solid material (0.72 g, 81%). $R_f = 0.7$ (10% MeOH in CH_2Cl_2, v/v). ^{1}H NMR ($CDCl_3$) δ 1.90 (3H, s, $COCH_3$), 3.07 (1H, dd, $J = 9.9$ Hz and 5.7 Hz, H-5′$_\alpha$), 3.19 (1H, dd, $J = 9.9$ Hz and 5.7 Hz, H-5′$_\beta$), 3.74, 3.81 (6H, s, $2 \times OCH_3$), 4.15 (1H, q, $J = 3.3$ Hz, H-4′), 4.67 (1H, q, $J = 5.4$ Hz, H-3′), 5.25 (1H, d, $J = 2.4$ Hz, H-2′), 5.85 (1H, d, $J = 2.4$ Hz, H-1′), 5.87 (1H, d, $J = 4.5$ Hz, OH-3′), 6.91 (4H, d, $J = 7.8$ Hz, Ar-DMT), 7.22–7.65 (13H, m, H-5, Ar-DMT and Ar-Bz), 8.01 (2H, d, $J = 7.5$ Hz, Ar-Bz), 8.22 (1H, d, $J = 7.5$ Hz, H-6) and 9.21 (1H, br s, NHCOPh). ^{13}C NMR ($CDCl_3$) δ 20.4 (COCH_3) 55.0 ($2 \times OCH_3$), 63.0 (C-5′), 73.9 (C-3′), 81.0 (C-2′), 85.4 (C-4′), 86.5 (C-1′), 91.0 (C(Ph)$_3$), 95.8 (C-5), 113.2 (Ar-DMT), 126.7, 127.6, 127.8, 128.4, 129.6, 132.7, 133.1, 135.3, 135.4, 144.7 (Ar-DMT and Ar-Bz), 146.2 (C-6), 154.4 (C-2), 158.1 (Ar-DMT), 163.4 (C-4), 167.2 (COPh), 169.1 (COCH_3). MALDI-HRMS m/z 714.2395 ([M + Na]$^+$, calculated for $C_{39}H_{37}N_3O_9Na$ 714.2421).

9-(2′-*O*-Acetyl-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)-2-*N*-isobutyrylguanine (23). The nucleoside **19** (0.50 g, 1.20 mmol) was co-evaporated with pyridine (2×10 mL) and then dissolved in a mixture of dichloromethane and pyridine (30 mL, 4:1). DMTCl (0.48 g, 1.43 mmol) was added and the resulting mixture was stirred for 12 h under an atmosphere of nitrogen. The reaction mixture was evaporated to dryness under reduced pressure and the residue was dissolved in dichloromethane (60 mL) whereupon washing was performed using a saturated aqueous solution of $NaHCO_3$ (2×20 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel (70–75% ethyl acetate in petroleum ether) to give nucleoside **23** as a white solid material (0.39 g, 45%). $R_f = 0.4$ (5% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (DMSO-d_6) δ 1.12 (6H, d, $J = 6.6$ Hz, COCH(CH_3)$_2$), 2.00 (3H, s, $COCH_3$), 2.78 (1H, sept, $J = 6.6$ Hz, COCH(CH$_3$)$_2$), 3.11–3.21 (2H, m, H-5′), 3.74 (6H, 2 s, $2 \times OCH_3$), 4.34 (1H, dd, $J = 10.2$ Hz and 4.8 Hz, H-3′), 4.53 (1H, dd, $J = 9.6$ and 5.1 Hz, H-4′), 5.54 (1H, t, $J = 4.2$ Hz, H-2′), 6.00–6.02 (1H, m, OH-1′ and OH-3′), 6.90 (4H, d, $J = 8.4$ Hz, Ar-DMT), 7.23–7.42 (9H, m, Ar-DMT), 8.24 (1H, s, H-8), 11.59 (1H, br s, NH), 12.12 (1H, br s, NHCOCH(CH$_3$)$_2$). ^{13}C NMR (DMSO-d_6) δ 18.8 (COCH(CH_3)$_2$), 20.4 (COCH_3), 34.7 (COCH(CH$_3$)$_2$), 55.0 ($2 \times OCH_3$), 62.5 (C-5′), 73.7 (C-3′), 81.6 (C-2′), 83.8 (C-4′), 85.4 (C-1′), 85.8 (C-Ph$_3$), 113.2 (Ar-DMT), 120.2 (C-5), 126.7, 127.6, 127.8, 129.7, 135.4, 135.4 (Ar-DMT), 138.2 (C-8), 144.7 (Ar-DMT), 148.1 (C-4), 148.3 (C-2), 154.7 (C-6), 158.1 (Ar-DMT), 169.6 (COCH_3), 180.1 (COCH(CH$_3$)$_2$). MALDI-HRMS m/z 720.2625 ([M + Na]$^+$, calculated for $C_{37}H_{39}N_5O_9Na$ 720.2639).

1-(2′-*O*-Acetyl-3′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)thymine (24). 2-Cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.36 mL, 0.16 mmol) was added dropwise to a stirred solution of the nucleoside **20** (0.90 g, 1.49 mmol) and anhydrous *N*,*N*-diisopropylethylamine (1.2 mL, 6.88 mmol) in anhydrous dichloromethane (40 mL) and the resulting mixture was stirred at RT for 5 h under an atmosphere of nitrogen. Methanol (2 mL), followed by dichloromethane (25 mL) were added and washing was performed using saturated aqueous $NaHCO_3$ (2×20 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography (35–40% ethyl acetate in petroleum ether, containing 0.5% Et_3N, v/v/v) over silica gel to give phosphoramidite **24** as a white solid material (0.93 g, 78%). $R_f = 0.51$, 0.53 (75% EtOAc in petroleum ether, v/v). ^{31}P NMR (121.5 MHz, $CDCl_3$) δ 152.44 and 153.08. MALDI-HRMS m/z 825.3216 ([M + Na]$^+$, calculated for $C_{42}H_{51}N_4O_{10}PNa$ 825.3235).

9-(2′-*O*-Acetyl-3′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)-6-*N*-benzoyladenine (25). The nucleoside **21** (0.80 g, 1.11 mmol) was co-evaporated with anhydrous acetonitrile (3 × 15 mL) and then dissolved in anhydrous dichloromethane (50 mL). Anhydrous *N,N′*-diisopropylethyl amine (1.6 mL, 9.18 mmol) was added followed by 2-cyanoethyl-*N,N′*-diisopropylphosphoamidochlorodite (0.30 mL, 1.34 mmol), and the resulting mixture was stirred for 3 h at RT under an atmosphere of nitrogen. Methanol (3 mL) followed by dichloromethane (35 mL) were added and successive washings were performed using saturated aqueous solutions of $NaHCO_3$ (2 × 35 mL) and brine (2 × 35 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel (50–60% ethyl acetate in petroleum ether, containing 0.5% Et_3N, v/v/v) to afford nucleoside **25** as a white solid material (0.78 g, 79%). $R_f = 0.50, 0.55$ (50% ethyl acetate in petroleum ether, v/v). ^{31}P NMR ($CDCl_3$) δ 151.95 and 152.76. MALDI-HRMS *m/z* 938.3582 ([M + Na]$^+$, calculated for $C_{49}H_{54}N_7O_9PNa$ 938.3612).

9-(2′-*O*-Acetyl-3′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)-4-*N*-benzoylcytosine (26). The nucleoside **22** (0.60 g, 0.87 mmol) was co-evaporated with anhydrous acetonitrile (3 × 10 mL) and then dissolved in anhydrous dichloromethane (30 mL). Anhydrous *N,N′*-diisopropylethyl amine (1.0 mL, 5.74 mmol) was added followed by 2-cyanoethyl-*N,N′*-diisopropylphosphoamidochlorodite (0.23 mL, 0.95 mmol) and the resulting mixture was stirred for 12 h at RT under an atmosphere of nitrogen. Additional 2-cyanoethyl-*N,N′*-diisopropylphosphoamidochlorodite (0.1 mL, 0.42 mmol) was added and stirring was continued for 5 h. Methanol (2 mL) followed by dichloromethane (20 mL) were added and successive washings were performed using saturated aqueous solutions of $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel (50–60% ethyl acetate in petroleum ether, containing 0.5% Et_3N, v/v/v) to afford nucleoside **26** as a white solid material (0.415 g, 80%). $R_f = 0.50, 0.55$ (50% EtOAc in petroleum ether, v/v). ^{31}P NMR ($CDCl_3$) δ 151.95 and 151.96. MALDI-HRMS *m/z* 914.3481 ([M + Na]$^+$, calculated for $C_{48}H_{54}N_5O_{10}PNa$ 914.3500).

9-(2′-*O*-Acetyl-3′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)-2-*N*-isobutyrylguanine (27). The nucleoside **23** (0.35 g, 0.49 mmol) was co-evaporated with anhydrous acetonitrile (3 × 10 mL) and then dissolved in anhydrous dichloromethane (30 mL). Anhydrous *N,N′*-diisopropylethyl amine (1.0 mL, 5.74 mmol) was added followed by 2-cyanoethyl-*N,N′*-diisopropylphosphoamidochlorodite (0.144 g, 0.6 mmol) and the resulting mixture was stirred for 12 h at RT under an atmosphere of nitrogen. Additional 2-cyanoethyl-*N,N′*-diisopropylphosphoamidochlorodite (0.1 mL, 0.42 mmol) was added and stirring was continued for 5 h. Methanol (2 mL) followed by dichloromethane (20 mL) were added and successive washings were performed using saturated aqueous solutions of $NaHCO_3$ (2 × 20 mL) and brine (2 × 20 mL). The organic phase was dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel (50–60% ethyl acetate in petroleum ether, containing 0.5% Et_3N, v/v/v) to afford nucleoside **27** as a white solid material (0.24 g, 50%). $R_f = 0.45, 0.50$ (50% EtOAc in petroleum ether, v/v). ^{31}P NMR ($CDCl_3$) δ 151.49 and 151.78. MALDI-HRMS *m/z* 920.3762 ([M + Na]$^+$, calculated for $C_{46}H_{56}N_7O_{10}PNa$ 920.3718).

2,2′-Anhydro-α-L-ribofuranosylthymine (28). The nucleoside **1** (2.00 g, 7.74 mmol) was dissolved in anhydrous *N,N′*-dimethylformamide (8 mL) followed by addition of diphenyl carbonate (4.14 g, 19.36 mmol) and sodium carbonate (0.19 g, 2.32 mmol), and the resulting reaction mixture was refluxed for 3 h whereupon it was cooled to RT and poured slowly into diethyl ether (200 mL) with stirring. The dark brown residue thus obtained was washed twice with diethyl ether (2 × 100 mL) and subjected to column chromatography on silica gel (14–18% methanol in chloroform, v/v) to afford nucleoside **28** as a white solid material (0.69 g, 32%). $R_f = 0.51$ (20% MeOH in $CHCl_3$, v/v). ^{1}H-NMR (DMSO-d_6): δ 1.95 (s, 3H, CH_3), 3.63–3.68 (m, 2H, H-5′$_{\alpha+\beta}$), 3.85 (br s, 1H, H-4′), 4.22 (m, 1H, H-3′), 5.28 (t, 1H, $J = 5.3$ Hz, H-2′), 6.24 (d, 1H, $J = 5.2$ Hz, H-1′), 7.70 (s, 1H, H-6). ^{13}C-NMR (75.5 MHz): δ 12.8 (CH_3), 59.9 (C-5′), 70.6 (C-3′), 81.2 (C-4′), 82.4 (C-2′), 90.0 (C-1′), 118.0 (C-5), 133.1 (C-6), 162.0 (C-4). MALDI-HRMS *m/z* 263.0641 ([M + Na]$^+$, calculated for $C_{10}H_{12}N_2O_5Na$ 263.0638).

2′-Azido-2′-deoxy-α-L-arabinofuranosylthymine (29). To a stirred solution of nucleoside **28** (0.80 g, 3.33 mmol) in anhydrous *N,N′*-dimethylformamide (16 mL) was added sodium azide (1.73 g, 26.7 mmol) and the resulting reaction mixture was heated under reflux for 9 h and then cooled to RT. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (4–5% methanol in chloroform, v/v) to afford the nucleoside **29** as a yellowish oil (0.58 g, 61%). $R_f = 0.25$ (5% MeOH in $CHCl_3$, v/v). ^{1}H-NMR (DMSO-d_6) δ 1.90 (s, 3H, CH_3), 3.62–3.76 (m, 2H, H-5′), 4.18–4.22 (m, 2H, H-2′ & H-3′), 4.31 (m, 1H, H-4′), 5.79 (br s, 1H, H-1′), 7.54 (s, 1H, H-6). ^{13}C-NMR (DMSO-d_6) δ 12.8 (CH_3), 62.6 (C-5′), 72.1 (C-2′), 75.6 (C-3′), 87.7 (C-4′), 90.5 (C-1′), 112.1 (C-5), 138.5 (C-6), 152.7 (C-4), 166.7 (C-2). MALDI-HRMS *m/z* 306,0795 ([M + Na]$^+$, calculated for $C_{10}H_{13}N_5O_5Na$ 306.0808).

2′-Amino-2′-deoxy-α-L-arabinofuranosylthymine (30). To a solution of nucleoside **29** (0.10 g, 0.35 mmol) in anhydrous methanol (4 mL) was added 10% Pd/C (10 mg) and the resulting reaction mixture was stirred for 8 h under an atmosphere of hydrogen. The mixture was filtered on a small bed of Celite and the bed was washed with 20 mL of hot methanol. The combined fractions after washing and filtration was evaporated to dryness under reduced pressure to afford nucleoside **4** as an off-white solid material (74 mg, 82%). $R_f = 0.32$ (20% MeOH in $CHCl_3$, v/v). ^{1}H-NMR (DMSO-d_6) δ 1.90 (s, 3H, CH_3), 3.62 (m, 1H, H-2′), 3.66–3.71 (m, 2H, H-5′), 4.13–4.17 (m, 1H, H-3′), 4.28 (br s, 1H, H-4′), 5.88 (d, 1H, $J = 3.6$ Hz, H-1′), 7.59 (s, 1H, H-6). ^{13}C-NMR (DMSO-d_6) δ 12.1 (CH_3), 61.4 (C-2′), 62.7 (C-5′), 75.8 (C-3′), 85.5 (C-4′), 89.4 (C-1′), 109.2 (C-5), 136.7 (C-6), 150.8 (C-4), 163.8 (C-2). MALDI-HRMS *m/z* 258.1090 ([M + H]$^+$, calculated for $C_{10}H_{16}N_3O_5Na$ 258.1084).

1-(2′-*N*-Trifluoroacetyl-α-L-arabinofuranosyl)thymine (31). To a suspension of nucleoside **30** (0.60 g, 2.33 mmol) in methanol (20 mL) was added 4-(*N*,*N*-dimethylamino)pyrimidine (0.16 g, 1.30 mmol) and ethyl trifluoroacetate (0.43 g, 3.03 mmol), and

the resulting reaction mixture was stirred for 5 h at RT. The solvent was removed under reduced pressure, and the residue was subjected to column chromatography on silica gel (0–10% methanol in dichloromethane, v/v) to afford nucleoside **31** as a white solid material (0.71 g, 87%). R_f = 0.5 (15% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (DMSO-d_6) δ 1.82 (3H, s, CH_3), 3.45 (1H, d, J = 11.4 Hz, H-5′$_\alpha$), 3.63 (1H, d, J = 11.7 Hz, H-5′$_\beta$), 4.17 (1H, m, H-4′), 4.30 (1H, m, H-3′), 4.49 (1H, q, J = 6.3 Hz, H-2′), 4.96 (1H, br s, OH-5′), 5.68 (1H, d, J = 5.7 Hz, OH-3′), 5.91 (1H, d, J = 7.8 Hz, H-1′), 7.73 (1H, s, H-6), 7.79 (1H, d, J = 5.4 Hz, NH-2′) and 11.29 (1H, s, NH). ^{13}C NMR (DMSO-d_6) δ 12.0 (CH_3), 59.9 (C-2′), 60.4 (C-5′), 70.7 (C-3′), 84.1 (C-4′), 84.8 (C-1′), 110.1 (C-5), 117.6 (CF_3), 136.1 (C-6), 150.6 (C-2), 156.5 ($COCF_3$) and 163.6 (C-4). MALDI-HRMS m/z 376.0727 ([M + Na]$^+$, calculated for $C_{12}H_{14}F_3N_3O_6Na$ 376.0726).

1-(2′-*N*-Trifluoroacetyl-5′-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)thymine (32). The nucleoside **31** (0.63 g, 1.78 mmol) was co-evaporated with anhydrous pyridine (2 × 5 mL) and then dissolved in a mixture of dichloromethane and pyridine (24 mL, 2:1) whereupon DMTCl (0.69 g, 2.05 mmol) was added. The resulting mixture was stirred for 14 h at RT under an atmosphere of nitrogen whereupon dichloromethane (100 mL) was added. The resulting mixture was washed with a saturated aqueous solution of $NaHCO_3$ (2 × 50 mL), dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by column chromatography over silica gel (20–80% ethyl acetate in petroleum ether, v/v) to afford nucleoside **32** (0.91 g, 78%) as a white solid material. R_f = 0.4 (5% MeOH in CH_2Cl_2, v/v). ^{1}H NMR (DMSO-d_6) δ 1.84 (3H, s, CH_3), 3.05 (1H, dd, J = 4.5 Hz, H-5′$_\alpha$), 3.16 (1H, br s, H-5′$_\beta$), 3.73 (6H, s, 2 × OCH_3), 4.38 (2H, m, H-3′ and H4′), 4.51 (1H, q, J = 7.5 Hz, H-2′), 5.77 (1H, d, J = 5.1 Hz, OH-3′), 5.99 (1H, d, J = 7.5 Hz, H-1′), 6.91 (4H, d, J = 8.7 Hz, ArH), 7.20–7.44 (9H, m, ArH), 7.78 (1H, s, H-6), 9.78 (1H, d, J = 7.8 Hz, NH-2′) and 11.35 (1H, s, NH). ^{13}C NMR (DMSO-d_6) δ 12.0 (CH_3), 55.0 (2 × OCH_3), 59.7 (C-2′), 62.7 (C-4′), 71.2 (C-3′), 81.8 (C-4′), 84.4 (C-1′), 85.3 ($C(Ph)_3$), 110.3 (C-5), 113.2 (Ar-DMT), 117.6 (CF_3), 126.6, 127.7, 127.8, 129.7, 135.5, 136.1, 144.8, 150.7, 158.0 (Ar-DMT, C-6 and C-2), 158.0 ($COCF_3$) and 163.6 (C-4). MALDI-HRMS m/z 678.2034 ([M + Na]$^+$, calculated for $C_{33}H_{32}F_3N_3O_8Na$ 678.2033).

1-(2′-*N*-Trifluoroacetyl-3′-*O*-(2-cyanoethoxy(diisopropylamino)phosphino)-5-*O*-(4,4′-dimethoxytrityl)-α-L-arabinofuranosyl)-thymine (33). The nucleoside **32** (0. 51 g, 0.77 mmol) was dissolved in anhydrous acetonitrile (20 ml) and *N*,*N*-diisopropylammonium tetrazolide (0.20 g, 1.16 mmol) and *bis*(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphine (0.61 ml, 1.94 mmol) were added. The resulting reaction mixture was stirred for 2 h at RT under an atmosphere of nitrogen whereupon it was diluted with EtOAc (100 mL), washed with H_2O (75 mL), washed with a 5% aqueous solution of $NaHCO_3$ (75 mL), washed with H_2O (75 ml), dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (20–60% EtOAc in petroleum ether, v/v) to afford nucleoside **33** (0.60 g, 90%) as a white solid material. R_f = 0.65 (80% EtOAc in petroleum ether, v/v); ^{31}P NMR ($CDCl_3$) δ 150.8 and 151.2. MALDI-HRMS m/z 878.3111 ([M + Na]$^+$, calculated for $C_{42}H_{49}F_3N_5O_9PNa$ 878.3112).

Synthesis of oligonucleotides containing α-ara nucleotide monomers. The oligomers (Table 1) were synthesized on an automated DNA synthesizer using the phosphoramidite approach. The synthesis of **ON9–ON20** containing three or six α-ara nucleotide monomers was performed in 0.2 μmol scale whereas the fully modified α-L-ara oligomers **ON21–ON24** were synthesized in 1.0 μmol scale on a universal support. Standard conditions of the synthesizer were used for incorporation of DNA monomers. The α-L-ara-, α-D-ara- and 2′-amino-α-L-ara phosphoramidite building blocks were used for the synthesis of modified oligomers **ON9–ON24**. The stepwise coupling yields for amidites **24–27** and **33** (10 min coupling time, 1*H*-tetrazole as activator for α-L-ara-T and α-D-ara-T monomers; 15 min coupling time, pyridinium hydrochloride as activator for α-L-ara-A and α-D-ara-A monomers) were 98–100% based on the absorbance of the dimethoxytriyl cation released after each coupling step. After detritylation, cleavage from the solid support and deacylation was carried out by using 30% aqueous ammonia solution (12 h, 55 °C). For fully modified oligomers, 2M methanolic ammonia was applied (30 min, 20 °C) followed by 30% aqueous ammonia (12 h, 55 °C). Analysis by ion-exchange HPLC verified the purity of all α-ara modified oligomers to be >80%, and their composition was verified by MALDI-TOF mass spectrometry (Table 1).

Thermal denaturation experiments. UV-based thermal denaturation experiments were performed on a Perkin-Elmer Lambda 35 UV/vis spectrometer equipped with PTP 6 (Peltier Temperature Programmer) in a medium or low salt buffer (see captions below tables for details). The solution of the two oligonucleotides (1.0 μM of each strand) were thoroughly mixed, heated to 90 °C and subsequently cooled to the starting temperature of the experiment (5 °C). Thermal denaturation temperatures (T_m values, °C) were determined as the maximum of the first derivative of the thermal denaturation curve (A_{260} *vs* temperature; reported T_m values are an average of two measurements within ±1.0 °C).

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